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UNIVERSITY OF ALBERTA

Recognition of Synthetic Oligosaccharide Analogs by the Blood-Group A, B and H Gene-Specified Glycosyltransferases

BY

©

Todd L. Lowary

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirement for the degree of **Doctor of Philosophy**

DEPARTMENT OF CHEMISTRY

Edmonton Alberta

Fall 1993

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Dedicated to the Memory of my

Maternal Grandfather:

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(1908-1988)

ABSTRACT

The A, B and H(O) blood-group antigens are biosynthetically assembled by three glycosyltransferases. The enzymes are, respectively, an α (1→3) N-acetylgalactosaminyltransferase (A-transferase) an α (1→3) galactosyltransferase (B-transferase) and an α (1→2) fucosyltransferase (H-transferase) The minimum acceptor for the fucosyltransferase encoded by the H-gene is β-D-Gal*p*-OR (**1**, R = octyl). The disaccharide α-L-Fuc*p*-(1→2)-β-D-Gal*p*-OR (**2**, R = octyl) is the minimum acceptor structure for the A and B-transferases. Eighteen analogs of both **1** and **2** have been chemically synthesized and enzymatically evaluated. These were analogs where the Gal OH-3, OH-4, and OH-6 were replaced, independently, with hydrogen, fluoro, O-methyl, amino, and acetamido functionalities. As well, analogs containing the 3 and 4 epimers of Gal and the C-5 dehydroxymethylated Gal derivative, L-arabinose, were prepared.

Evaluation of the monosaccharides with a cloned H-transferase indicated that the enzyme will accept, as substrates, the C-6 modified derivatives. The C-3 modified Gal derivatives are, to varying degrees, inhibitors of this enzyme. The Gal OH-4 modified compounds showed low activity as both acceptors and inhibitors suggesting that this hydroxyl group is important for recognition.

The testing of the disaccharides with both the A- and B-transferases from human serum indicates that both enzymes will tolerate modifications at both the Gal C-6 and C-3 positions. The C-6 modified derivatives, except the *arabino* and acetamido derivatives, are substrates with K_m values between 5 and 565 μM. The C-3 modified derivatives were inhibitors with calculated or estimated K_i values of 0.2 to 313 μM. The 3-amino compound is a very potent inhibitor of both enzymes, possessing an estimated K_i of 200 nM for the A-transferase and 5 μM for the B-transferase. All of the Gal OH-4 modified derivatives are completely inactive, both as substrates and inhibitors, indicating that this hydroxyl group is critical for enzymatic recognition.

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LIST OF ABBREVIATIONS

Abbreviation	Name	Abbreviation	Name
ABP	arabinose binding protien	His	histidine
Ac	acetate	IR	infrared
Ala	alanine	Leu	leucine
All	allyl	Lys	lysine
Ara	arabinose	Man	mannose
Arg	arginine	Me	methyl
Asp	aspartate	Met	methionine
ATP	adenosine triphosphate	NDP	nuceloside diphosphate
Bn	benzyl	NeuAc	9-acetyl neuraminic acid, sialic acid
Bz	benzoyl	NMR	nuclear magnetic resonance
CMP	cytosine monophosphate	Ph	phenyl
DAST	diethylamino sulfur trifluoride	Phe	phenylalanine
DPM	disintigrations per minute	Phth	phthaloyl
FABMS	Fast atom bombardment mass specromtry	RER	rough endoplasmic reticulum
Fuc	fucose	Ser	serine
Gal	galactose	Tf	trifluoromethanesulfonyl
GalNAc	N-acetylgalactosamine	Thr	threonine
GBP	glucose binding protein	TLC	thin-layer chromotography
GDP	guanosine diphosphate	Trp	tryptophan
gem	geminal	Ts	p-toluenesulfonyl
Glc	glucose	Tyr	tyrosine
GlcNAc	N-acetylglucosamine	UDP	uridine diphosphate
Gly	glycine	vic	vicinal
GPI	glycerol phosphatidyl inositol	Xyl	xylose
GS4	*griffonia simplicifolia* lectin 4		

LIST OF ABBREVIATIONS-CONTINUED

AMINO ACID ABBREVIATIONS USED IN FIGURES 16 AND 17

One letter code	Amino acid	One letter code	Amino acid
A	alanine	M	methionine
C	cysteine	N	asparagine
D	aspartate	P	proline
E	glutamate	Q	glutamine
F	phenylalanine	R	arginine
G	glycine	S	serine
H	histidine	T	threonine
I	isoleucine	V	valine
K	lysine	W	tryptophan
L	leucine	Y	tyrosine

CHAPTER ONE:

INTRODUCTION

1. 1 GENERAL INTRODUCTION

It is now widely recognized that carbohydrates fulfill a number of biologically important roles aside from the energy storage and structural functions classically assigned to them. Over the past twenty-five years it has been established that all mammalian cells have a layer of carbohydrate structures on their surfaces[1-5]. Many reports over the last two decades have documented that these carbohydrates serve as important recognition factors for a number of cellular processes, both normal and abnormal[6-8]. Normal recognition processes include cell-cell recognition[9], the binding of the sperm to the egg during fertilization[10,11], the development of neurons[12,13] and the binding of viruses[14-17] and bacteria[18-19]. Perhaps of more interest, is the role these carbohydrates play in the development and metastasis of cancer[20-25] that has sparked much investigation. It has been shown that during carcinogenesis aberrant oligosaccharides are produced on cell surfaces[26-28]. As well, levels of glycosyltransferases, the enzymes responsible for the biosynthesis of carbohydrates in the body, are elevated in cells from a number of cancerous tissues[26-28]. Most recently, interest in glycoscience has been bolstered by the demonstration that oligosaccharides serve as recognition factors for molecules which direct lymphocytes to sites of bodily injury [29-33]. These cell-adhesion molecules (CAM), also known as selectins, are important mediators in the early stages of inflammation.

1. 2. TYPES OF CELL SURFACE OLIGOSACCHARIDES COMMONLY FOUND IN MAMMALIAN SYSTEMS

The polyhydroxylated structures of carbohydrates enable them to form not only linear, but also branched sequences. This branching ability, not normally found in other important biopolymers, makes possible the formation of structures with enormous diversity. So while three different amino acids are able to form only six tripeptides, 1056 unique trisaccharides are possible from the joining of 3 different monosaccharide residues[34]. As a result of this enormous structurally diversity, carbohydrates are ideally suited to serve as recognition factors. While the possibilities for the structure of the oligosaccharides are many, the way in which carbohydrates are linked to cell surfaces can be divided into two groups. To date, all cell-surface oligosaccharide structures reported have been linked covalently to either membrane associated proteins or lipids.

1. 2. 1 PEPTIDE-CARBOHYDRATE LINKAGES

Of those carbohydrates linked to proteins, the vast majority are either N-linked, through an amide linkage to asparagine, or O-linked, via a linkage to the hydroxyl group of serine or threonine[35]. Other, less frequent carbohydrate-protein linkages include those involving the sulfhydryl group of cysteine[34] and the hydroxyl groups of tyrosine[36] and hydroxylysine[34].

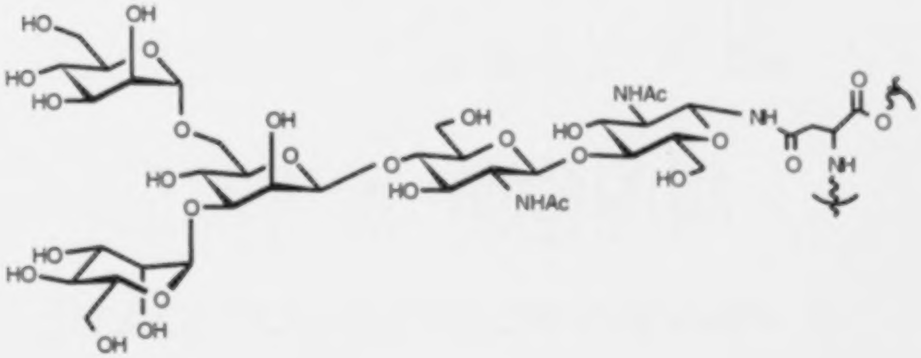
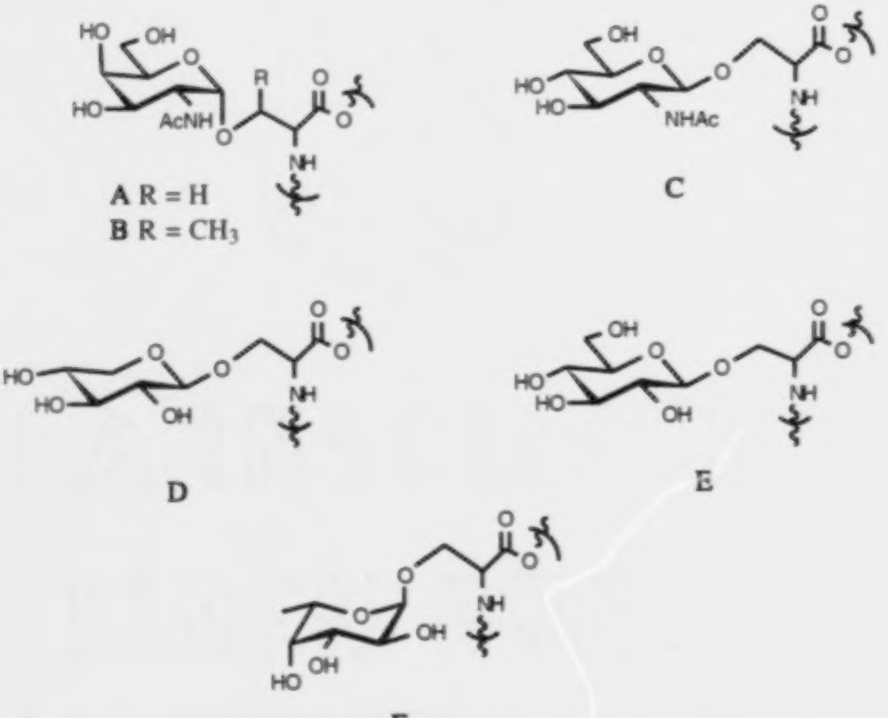


Fig 1. Pentasaccharide core common to N-linked glycoproteins.

All N-linked oligosaccharides found on cell surfaces have a common pentasaccharide core structure: Man α-(1→6)-[Man -α -(1→3)-]-Man-β-(1→4)-GlcNAc-β-(1→4)-GlcNAc-β-Asn (Fig. 1)[35]. A consensus sequence for N-glycosylation has been established. Only the asparagines in the amino acid sequence Asn-X-Ser/Thr, where X can be any amino acid except proline, are glycosylated[37]. In a given protein, however, not all asparagines in this sequence are glycosylated. The factors governing which asparagines are glycosylated and which are not are still not completely understood. Originally it was suggested that some asparagines are hidden by the rapid folding of the protein, thus making them inaccessible to the enzyme that transfers the initial carbohydrates to the protein[35]. More recently the proposal has been advanced that the ability to adopt a reactive conformation is also important[38-41].



A R = H
B R = CH_3

C

D

E

F

Fig 2. Examples of O-linked glycoprotein precursors.

For O-linked glycosylation no consensus sequence has been established, although a number of reports indicate that the serine or threonine that is glycosylated is found often, but not always, in a proline-rich region of the protein[42-46]. As well, O-glycans differ from N-glycans in that they do not possess a common core structure. Instead a number of carbohydrate-protein linkages have been reported. Among them are the GalNAc-α-Ser/Thr (Fig 2 A, B)[47], GlcNAc-β-Ser (Fig 2 C)[48], Xyl-β-Ser (Fig 2 D)[34], Glc-β-Ser (Fig 2 E)[49], and Fuc-α-Ser (Fig 2 F)[49] linkages. More complicated structures are then built up from these monosaccharide precursors. Of these linkages the most common one is the first, the GalNAc-α-Ser linkage.

1. 2. 2 LIPID-CARBOHYDRATE LINKAGES

Carbohydrates linked to lipids embedded in the cell membrane can be divided into two distinct groups, those linked to ceramide and those linked to phosphoglycerol derivatives. The most commonly found structures are those attached to ceramide via a β-linked lactosyl carbohydrate unit (Fig 3)[50]. These structures form the basis for all glycosphingolipids, including the gangliosides, sialic acid containing glycosphingolipids found primarily in the brain.

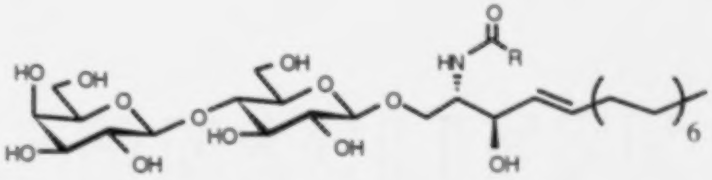


Fig 3. Lactosyl-ceramide linkage found in glycolipids.
R = long chain alkyl groups

The second group, discovered rather recently in comparison with the first, forms part of a linking arm for the attachment of proteins to membranes[51-54]. These glycosyl phosphatidyl inositol (GPI) anchors consist of a phosphoglycerol unit with two long chain alkyl or acyl groups on the other two, non-phosphorylated, glycerol hydroxyl groups. The long hydrophobic chains are embedded in the cell membrane. From the phosphate group the carbohydrates are attached via a six membered ring hexitol, inositol. The structures of the oligosaccharide vary, but appear to always have a core structure of glucosamine and three mannose residues. The protein is attached to the carbohydrate through an ethanolamine-phosphate linkage with a hydroxyl group at the non-reducing end of the oligosaccharide (Fig 4).

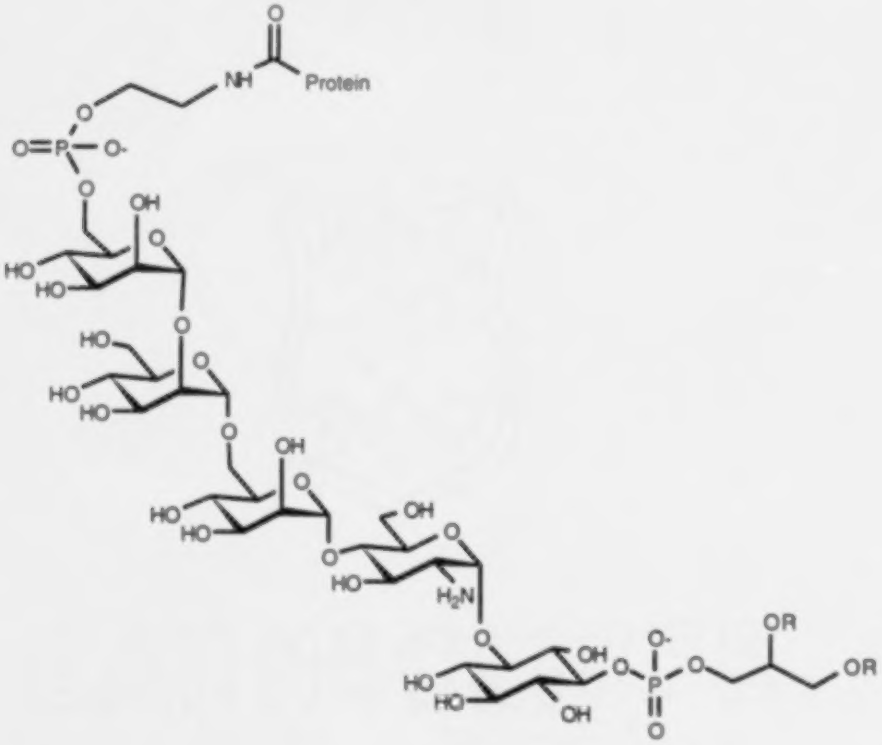


Fig 4. Core common to GPI anchors, R = long chain ester or alkyl groups.

1.3 BIOLOGICAL FORMATION OF OLIGOSACCHARIDES

The biosynthesis of oligosaccharides attached to the surfaces of mammalian cells is controlled by the combined action of two classes of enzymes, the glycosyltransferases and the glycosidases[35]. These classes of enzymes have complementary functions: the former enzymes add carbohydrates to oligosaccharide chains and the latter cleave them from chains.

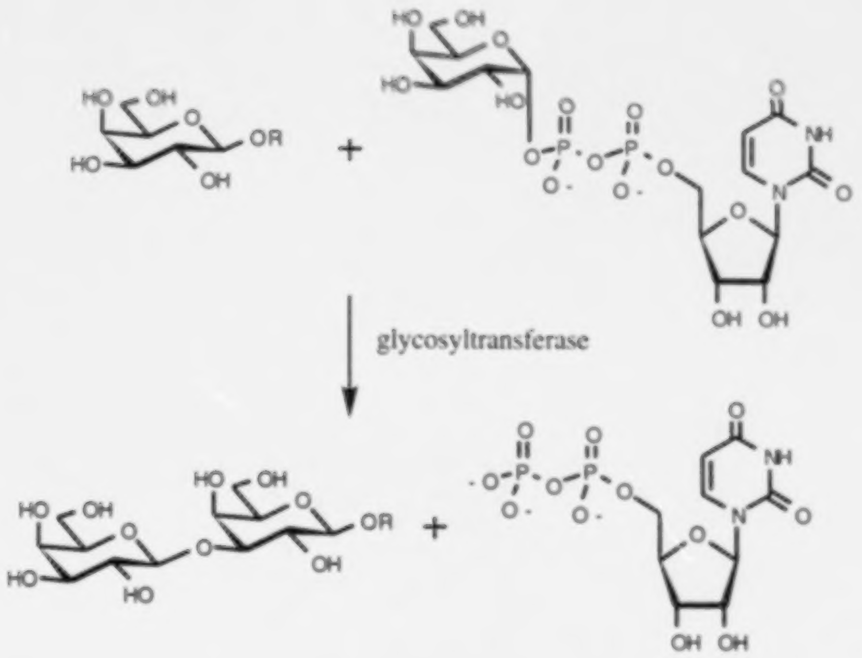


Fig 5. General Scheme for glycosyltransferase reaction. Shown here for the transfer of galactose from UDP-Gal. R = glycoconjugate.

Glycosyltransferases catalyze the transfer of carbohydrates from activated carbohydrate donors to a hydroxyl group at the non-reducing end of a growing oligosaccharide (Fig 5)[55]. The activated carbohydrate donors *in vivo* are nucleotide sugars. In humans, the sugar nucleotides used are derivatives of either guanine (GDP-Fuc, GDP-Man), uridine (UDP-Gal, UDP-Glc, UDP-GlcNAc, UDP-GalNAc, UDP-Xyl) or cytosine (CMP-NeuAc)[56]. Glycosidases remove carbohydrate units from oligosaccharides, producing in most cases a reducing monosaccharide and a shortened oligosaccharide (Fig 6)[57]. An interesting glycosidase has recently been isolated from the leech *Macrobella decora*[58]. This sialidase removes sialic acid from oligosaccharides, providing the shortened glycoconjugate and a 2,7-anhydro derivative of sialic acid, not free sialic acid.

The formation of glycoproteins is a largely a post-translational event carried out in both the rough endoplasmic reticulum (RER) and the Golgi apparatus[35].

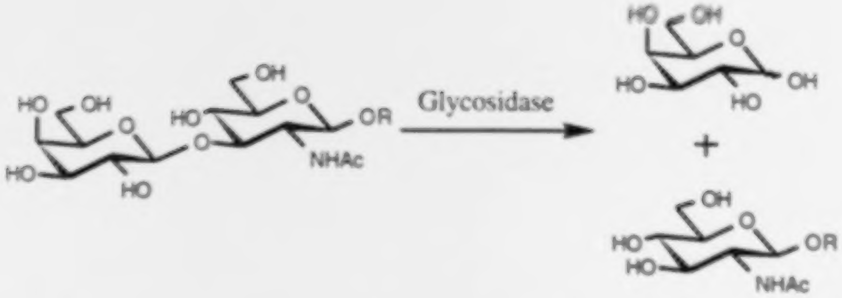


Fig 6. β-Galactosidase hydrolysis of a terminal galactose disaccharide. R = glycoconjugate.

Glycosylation of lipid precursors takes place in the Golgi[50]. The biosynthesis of these oligosaccharides, especially those N-linked in proteins, is a complex process which has been the subject of a number of reviews[35, 50, 59-62]. A brief overview is presented below.

1. 3. 1 N-LINKED OLIGOSACCHARIDES

The biosynthesis of N-linked structures[35] begins with the assembly of a large oligosaccharide linked to a lipid precursor, dolichol phosphate. After formation of this molecule the oligosaccharide is transferred, as a unit, to asparagine residues on the protein. This step occurs in the RER, and may occur during translation[63]. A number of glycosidases then act on this glycoprotein, both in the RER and Golgi, sequentially removing carbohydrates to provide structures that are then acted upon by the various glycosyltransferases in the Golgi. In this manner, the wide array of cell-surface oligosaccharides found are produced (Fig 7). The nascent glycoproteins then leave the Golgi and are transported to the cell surface by vesicles.

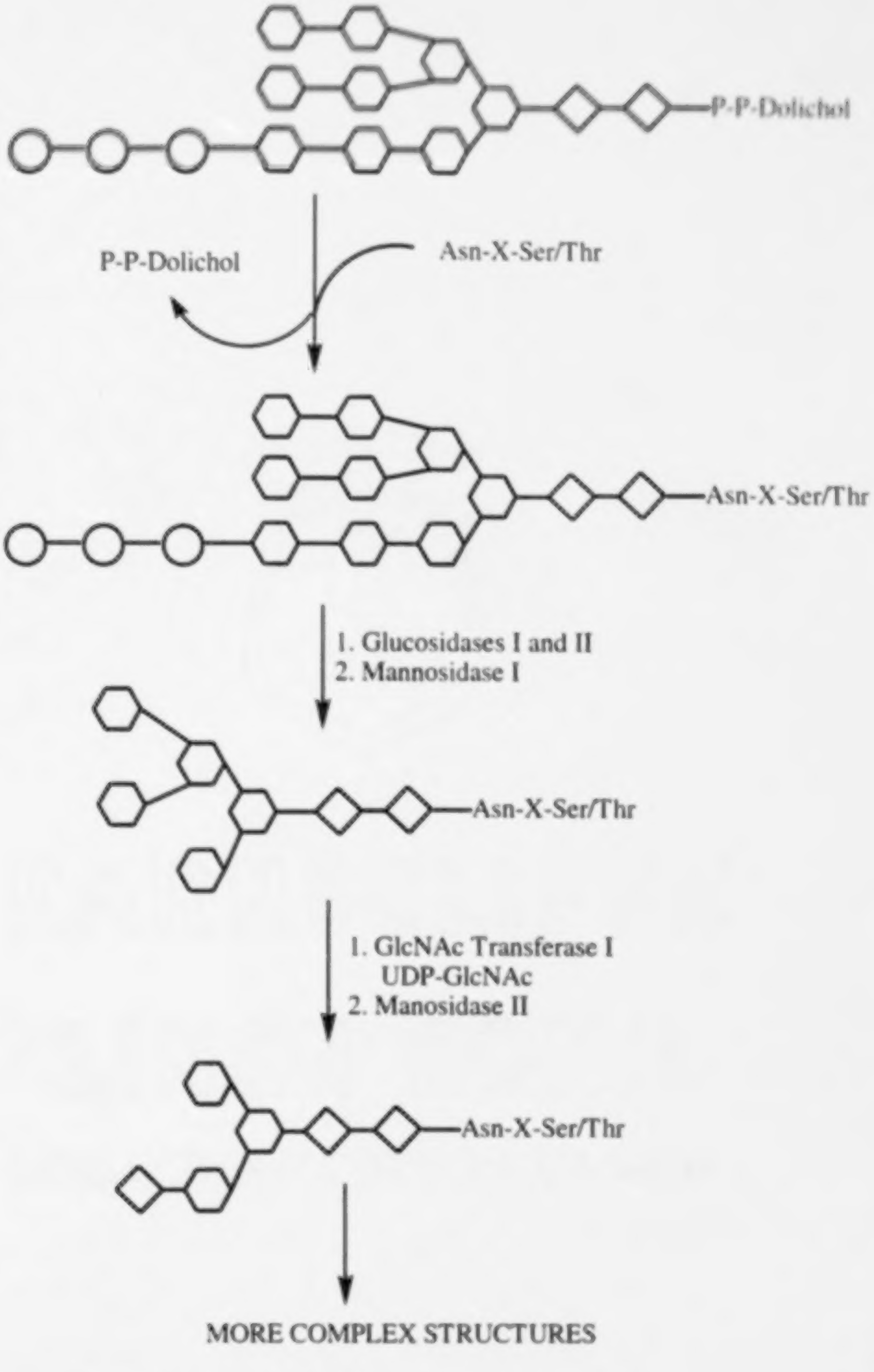

P-P-Dolichol
P-P-Dolichol
Asn-X-Ser/Thr
Asn-X-Ser/Thr
1. Glucosidases I and II
2. Mannosidase I
Asn-X-Ser/Thr
1. GlcNAc Transferase I
UDP-GlcNAc
2. Manosidase II
Asn-X-Ser/Thr
MORE COMPLEX STRUCTURES

Fig 7. Early steps in the biosynthetic pathway of N-glycans. Diamond = GlcNAc, Hexagon = Man, Circle = Glc.

1. 3. 2 O-LINKED OLIGOSACCHARIDES AND GLYCOLIPIDS

O-glycans and ceramide based glycolipids are synthesized by the sequential action of glycosyltransferases in the Golgi[50, 60-62], building up the oligosaccharides one monosaccharide unit at a time (Figs 8, 9). The biosynthetic pathway of GPI anchors in humans is unknown, although it has been suggested[51] that it is probably similar to their biosynthesis in the parasite *Trypanosoma brucei.* The biosynthesis in that organism, like that of N-glycan synthesis, involves a number of modifications on the original core before the final expressed oligosaccharide is produced[64].

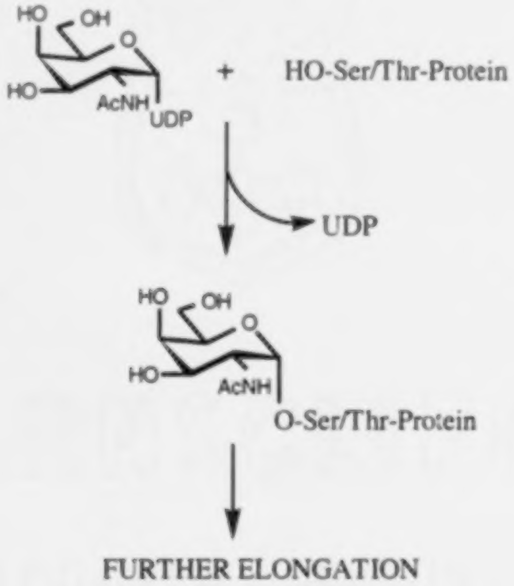
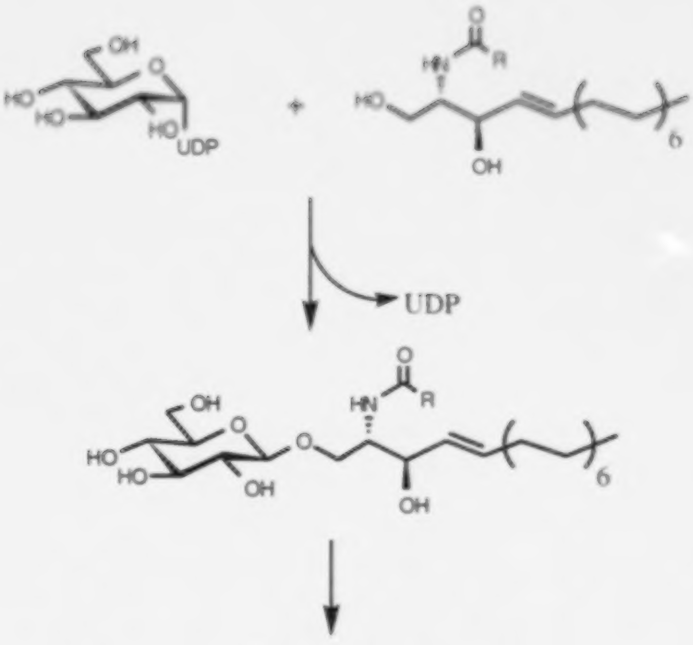


Fig 8. Formation of carbohydrate-peptide linkages in O-linked glycans.

1. 3. 3 INHIBITORS OF GLYCOSYLTRANSFERASES

The major interest of our research group, and the subject of this thesis, is the development of glycosyltransferase inhibitors. Up until about five years ago, and even today, most of the studies involving inhibition of glycosylation have involved the inhibition of either the glycosidases that act early in the biosynthetic pathway or the



FURTHER ELONGATION

Fig 9. Formation of carbohydrate-ceramide linkage in glycosphingolipids. R = long chain alkyl group.

enzyme that transfers the carbohydrate from the dolichol-carbohydrate precursor to the protein[65-67]. The result is either cells with glycosylation patterns drastically different from those that occur naturally on cells, or cells that lack glycosylation altogether.

The development of specific glycosyltransferase inhibitors that inhibit enzymes that add terminal sugars has been hampered by a number of factors, among them a lack of knowledge of substrate specificity of the enzymes, and the time involved in synthesizing carbohydrate analogs via organic synthesis to probe this specificity[68, 69]. More recently, in part due to the discovery that these enzymes will recognize structures considerably smaller than their natural structures (see for example reference 70), examples of glycosyltransferase inhibitors acting on enzymes which act late in the pathway have been prepared[70-73]. For most of the enzymes studied, it has been possible to replace large oligosaccharide structures (>5 monosaccharide units) with smaller fragments of the those structures (di- and trisaccharides) and still maintain activity. The use of these smaller fragments makes the synthesis of a number of substrate analogs much simpler.

As mentioned previously, the expression of carbohydrate structures has been reported to differ between cancerous and normal cells. Since the glycosyltransferases are responsible for biological glycosidic bond formation, it is likely that the activity of these enzymes is modulated in cancerous cells. Indeed, as mentioned at the beginning of this work, the levels of glycosyltransferase activity have been shown to be altered in cancerous tissues, and for this reason compounds that act as inhibitors of these enzymes are of interest. Our work in this area has both theoretical and practical significance, in that specific glycosyltransferase inhibitors can serve both as tools for studying the effects of modified cell glycosylation and as potentially useful anti-cancer drugs.

1. 3. 4 SUGGESTED GLYCOSYLTRANSFERASE MECHANISMS

During the transfer of the carbohydrate from a sugar nucleotide catalyzed by the glycosyltransferase, the stereochemistry of the anomeric center of the sugar nucleotide can be preserved, that is, the enzyme transfers the carbohydrate with retention of configuration, or inverted. The majority of glycosyltransferases proceed with inversion of configuration. Although no precise mechanisms are known, hypotheses exist.

The simplest mechanism to explain inversion of configuration is a direct S_N2-type displacement of the nucleoside diphosphate (NDP) from the sugar nucleotide by the acceptor hydroxyl group[74]. Possible assistance by a base on the enzyme to help deprotonate the incoming alcohol has also been proposed (Fig 10).

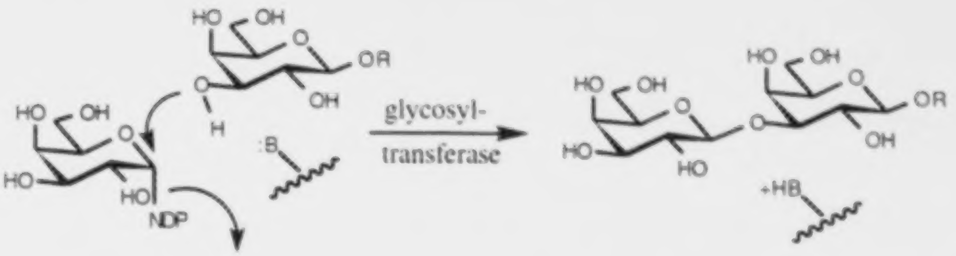

HO
OH
O
OR
OH
H
HO
OH
O
HO
HO
NDP
:B
glycosyl-
transferase
HO
OH
O
HO
HO
O
HO
OH
O
OR
OH
+HB

Fig 10. Proposed mechanism for the glycosyltransfase mediated transfer of a carbohydrate with inversion of configuration. R = Glycoconjugate.

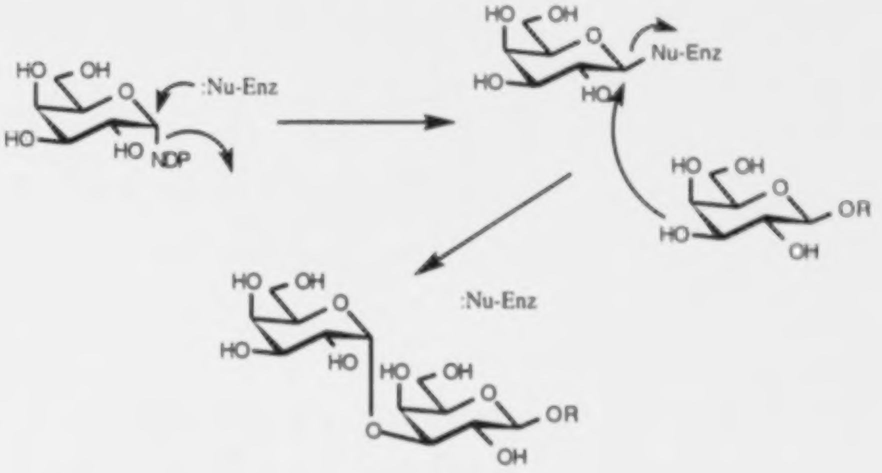

HO
OH
O
:Nu-Enz
HO
HO
NDP
HO
OH
O
Nu-Enz
HO
HO
HO
OH
O
OR
HO
OH
:Nu-Enz
HO
OH
O
HO
HO
HO
OH
O
OR
O
OH

Fig 11. Proposed mechanism for the glycosyltransfase mediated transfer of a carbohydrate with retention of configuration. R = Glycoconjugate.

To explain retention, a double displacement mechanism, similar to those proposed for certain glycosidases[74,75] seems likely. The first step would be the displacement of the NDP from the sugar nucleotide by a nucleophile located in the active site thus forming a covalent glycosyl-enzyme intermediate. Transfer of the sugar residue to the growing oligosaccharide would then be completed by expulsion of the enzyme from the glycosyl-enzyme intermediate by the OH of the acceptor residue (Fig 11). While the inversion mechanism does not necessarily rely on the presence of an enzymatic base, the retention mechanism requires the presence of a nucleophile.

The hypothesized presence of an essential nucleophile on the enzyme provides a target for possible enzyme deactivation via covalent reaction with an acyl, alkyl or other group.

1. 3. 5 GLYCOSYLTRANSFERASES STUDIED IN THIS THESIS

The glycosyltransferases studied in this thesis are those responsible for the biosynthesis of the A, B and O(H) blood-group antigens. The O (or H) antigenic determinant is the disaccharide α-L-Fuc*p*-(1→2)-β-D-Gal*p*-OR. The trisaccharides α-D-Gal*p*NAc-(1→3)[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-OR and α-D-Gal*p*-(1→3)[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-OR, are respectively the A and B blood group determinants[76]. In all cases R is a glycoconjugate. Fig. 12 shows the biosynthetic pathway for these antigens. The O antigen is synthesized by the addition of fucose from GDP-fucose to the 2 position of glycoconjugates possessing a terminal β-linked galactose residue. This transfer occurs with inversion of configuration. Subsequent formation of the A and B antigens proceeds with retention of configuration, and is completed by the transfer of either N-acetylgalactosamine or galactose from their corresponding UDP derivatives to the galactosyl 3 hydroxyl group of the O disaccharide antigen[76]. The enzymes responsible for these transfers are an α (1→2) fucosyltransferase (H-enzyme or Se-enzyme EC 2.4.1.69), an α (1→3) N-acetylgalactosaminyltransferase (A-transferase, EC 2.4.1.40), and an α (1→3) galactosyltransferase (B-transferase, EC 2.4.1.37)[47,76].

1. 4 OCCURRENCE OF A, B AND O ANTIGENS

The A, B and O antigens are ubiquitous biological oligosaccharide structures. Though originally described as constituents on the surfaces of red blood cells in 1900 by Landsteiner[77], they have also been shown to be present in a wide range of other tissue cells and in soluble form in the cytoplasm[78]. It is now known that they serve as important antigens in fetal development and organ differentiation, in addition to their well-known critical importance in blood transfusions and transplant operations[79].

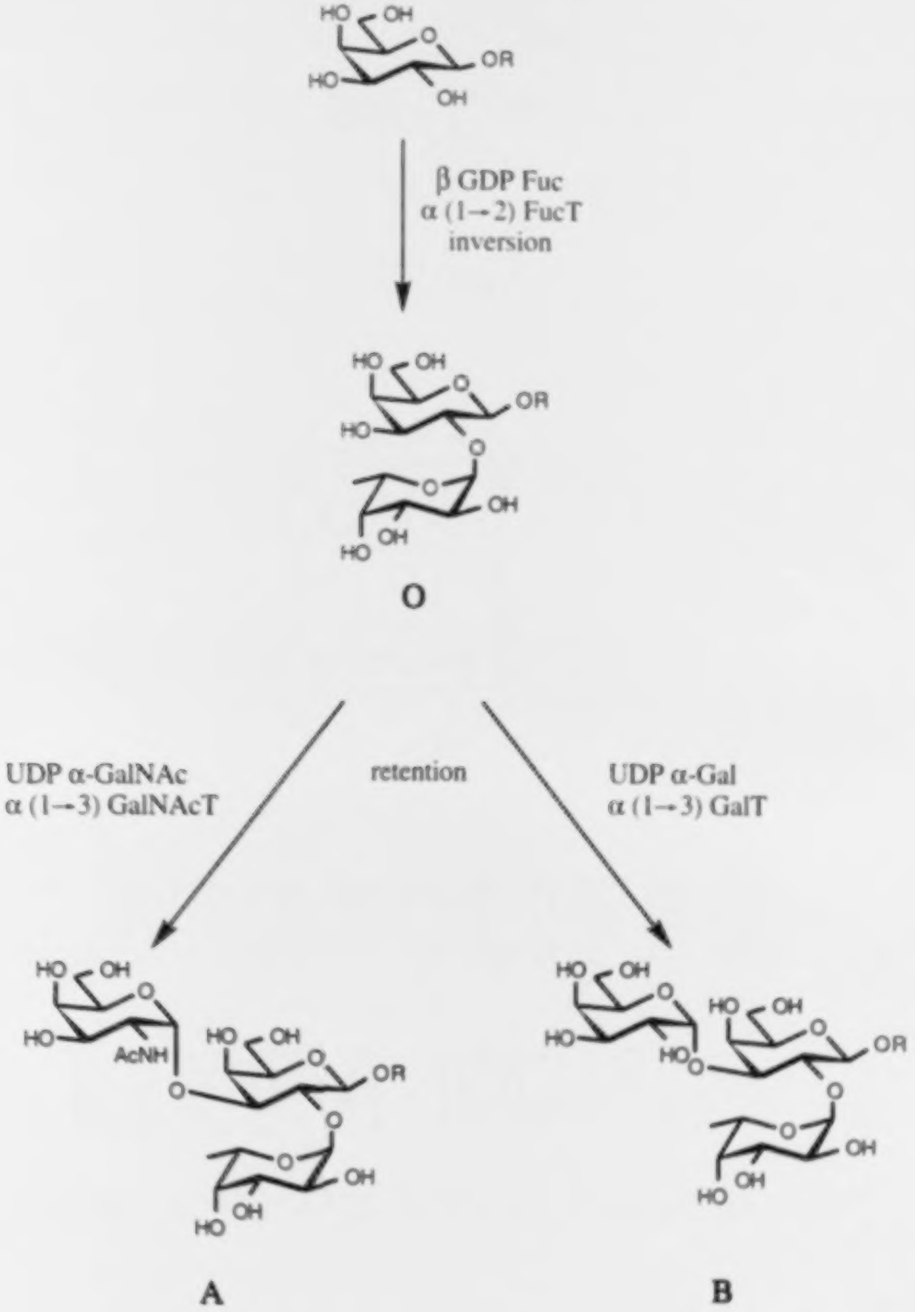

β GDP Fuc
α (1→2) FucT
inversion
O
UDP α-GalNAc
α (1→3) GalNAcT
retention
UDP α-Gal
α (1→3) GalT
A
B
OR
OH
HO
AcNH

Fig 12. Biosynthesis of the A, B and O blood group antigens.
R = glycoconjugate.

Recently, these structures have been discovered on Von Willebrand Factor, a glycoprotein involved in the blood clotting process. However, their exact role on this protein is unknown[80]. Like other carbohydrate antigens, the expression of these structures on cell surfaces is modulated during the onset of cancer[81-92]. The widespread occurrence of these carbohydrate antigens as well as the specific reports that levels of similar α (1→3) Gal-transferases are elevated in patients with Ehrlich carcinoma[93] and in mouse teratocarcinoma[94] led us to initiate this study.

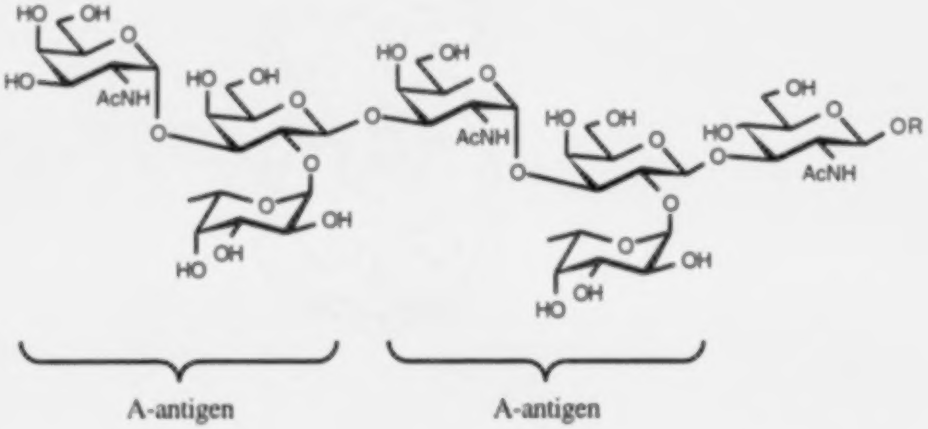


Fig 13 A. Structure of human glycolipid oligosaccharide containing two sequentially linked A-determinants

These antigens have been shown to occur most often at the non reducing termini of both glycoproteins (N and O linked), as well as glycolipids. It is known that the enzymes that synthesize these structures will act on all glycoconjugates possessing the appropriate precursor structure at their non-reducing terminus. Until about 10 years ago, it was believed that these structures occurred only at the terminal ends of oligosaccharides and that these antigens acted as chain terminating structures. Since then, however, these structures have been discovered as internal components of oligosaccharide chains. One, a human glycolipid from A erythrocytes, has been shown to have two A antigens linked sequentially via the GalNAc residue (Fig 13A)[95]

This structure, termed repetitive A, has been shown to be a major constituent of A antigens located on erythrocytes. Another has more recently been found in the epithelial cells of inbred rats[96]. This structure has two A antigens linked via a GlcNAc residue which is in turn linked through an α (1→6) linkage to the 6 position of the Gal residue (Fig 13 B).

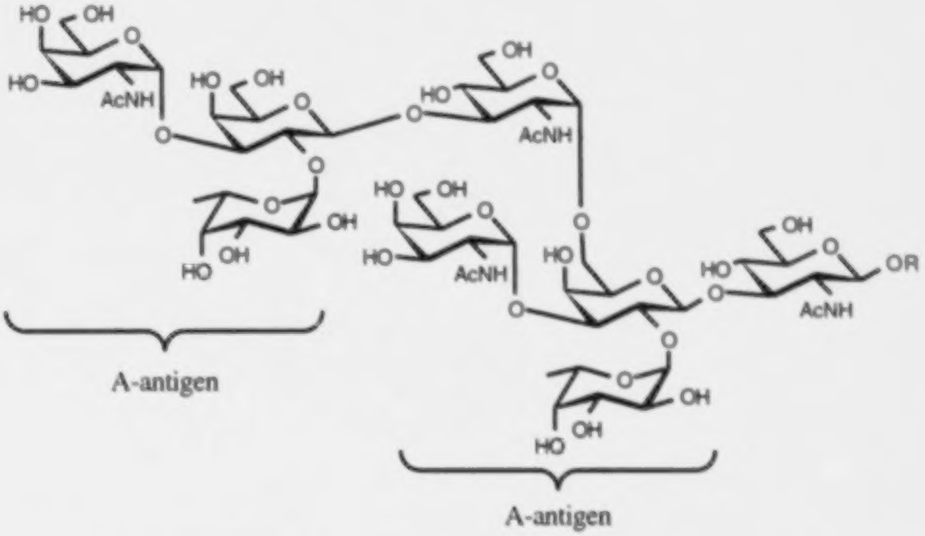


Fig 13 B. Structure of glycolipid oligosaccharide from inbred rats containing two A-determinants linked in tandem.

For the fucosyltransferase that synthesizes the O antigen the minimum structure requirement is a β-linked galactose residue at the non reducing end of an oligosaccharide. This galactose however can be linked to either GlcNAc, GalNAc, Gal or Glc through either the 3 or 4 hydroxyl group. To date the A, B and O blood group antigens have been shown to occur on the six different disaccharide structures shown below. (R = glycoconjugate)[97]:

Type I	Gal-β-(1→3)-GlcNAc-β-1→R
Type II	Gal-β-(1→4)-GlcNAc-β-1→R
Type III	Gal-β-(1→3)-GalNAc-α-1→R
Type IV	Gal-β-(1→3)-GalNAc-β-1→R

Type V Gal-β-(1→3)-Gal-β-1→R

Type VI Gal-β-(1→4)-Glc-β-1→R

The occurrence of a specific type of chain has been shown, at least to some extent, to be tissue and glycoconjugate specific. For example, Type I chains are found mainly in endodermal tissues and Type 2 chains are found primarily on erythrocytes. Both Type I and Type II structures are found on both glycoproteins (N and O-linked) and lipids. Type III structures occur predominantly O-linked to proteins and Type IV only on lipids[86]. Notice that the repetitive A structure (Fig 13A) contains both Type II and Type III linkages; the former being in the internal determinant, the latter in the terminal structure.

1.5 CONTROL OF BIOSYNTHESIS

Early in this century, the A and B antigens were proposed to be inherited following simple Mendelian genetics, with the A and B genes being dominant over the O gene. In 1924, Bernstein proposed a three-allelic model for inheritance of these genes[98]. Thus an individual was A if his genotype was AO or AA, and B if the genotype was BB or BO. AB individuals could have only one genotype, AB. Similarly, O individuals possessed an OO genotype.

Much later, in the late 1950's to late 1960's[99-102], A individuals were shown to express an α (1→3) N-acetylgalactosaminyltransferase (A-transferase), B individuals an α (1→3) galactosyltransferase (B-transferase). AB individuals had both enzymes and O individuals had neither. It was therefore proposed that the antigens were secondary gene products, the primary gene products being the enzymes that added the required sugars, e.g., the A and B genes encoded for the A and B transferases. The O gene was either thought to be silent, or produced a protein that was inactive[103]. Two types of AB individuals are known, those which possess both enzymes and those, termed cis-AB[104], who have only one enzyme capable of producing both structures.

Furthermore, A individuals can be classified into two different subgroups, A_1 and A_2[76]. The groups were identified over 50 years ago[105] and it is now believed that there are two distinct genes coding for two different enzymes[106]. The distinction between these enzymes has been the subject of much investigation. Cells from the two individuals differ in that A_1 individuals are able to synthesize more antigens than are A_2 individuals[76]. As well it has been shown that the A_2 transferase is less efficient than the A_1 transferase[103]. For many years it was unknown whether there was a qualitative difference between cells from the two subgroups. However, with the development of monoclonal antibodies that could recognize, for example, Type I versus Type II structures, the picture slowly became more clear[86].

It is now known that the repetitive A structures mentioned above (Fig 13A), are found primarily in A_1 individuals[107]. Moreover, the most recent work suggests that the two enzymes differ in their ability to recognize Type III chains. The differences are further explained below and in Fig 14[86]. Starting from a Type II A-determinant (A-Type II), the first two steps in forming a repetitive A structure are the addition of Gal to the GalNAc and then Fuc to Gal to form an oligosaccharide with a terminal Type III O-determinant (O-Type III). It is believed that both A_1 and A_2 individuals are equally able to prepare O-Type III from A-Type II. At this point, the differences between the two transferases manifest themselves. The A_1-transferase, which efficiently transfers GalNAc to the O-Type III, forms the repetitive A structure. The A_2-transferase is inefficient in recognizing O-Type III structures and hence a build up of the O-Type III occurs. Indeed, the accumulation of O-Type III antigens is observed in A_2 individuals[108]. It remains to be seen whether or not this is the only qualitative difference between individuals from these A subgroups. Furthermore, the differences in the enzymes themselves are not known.

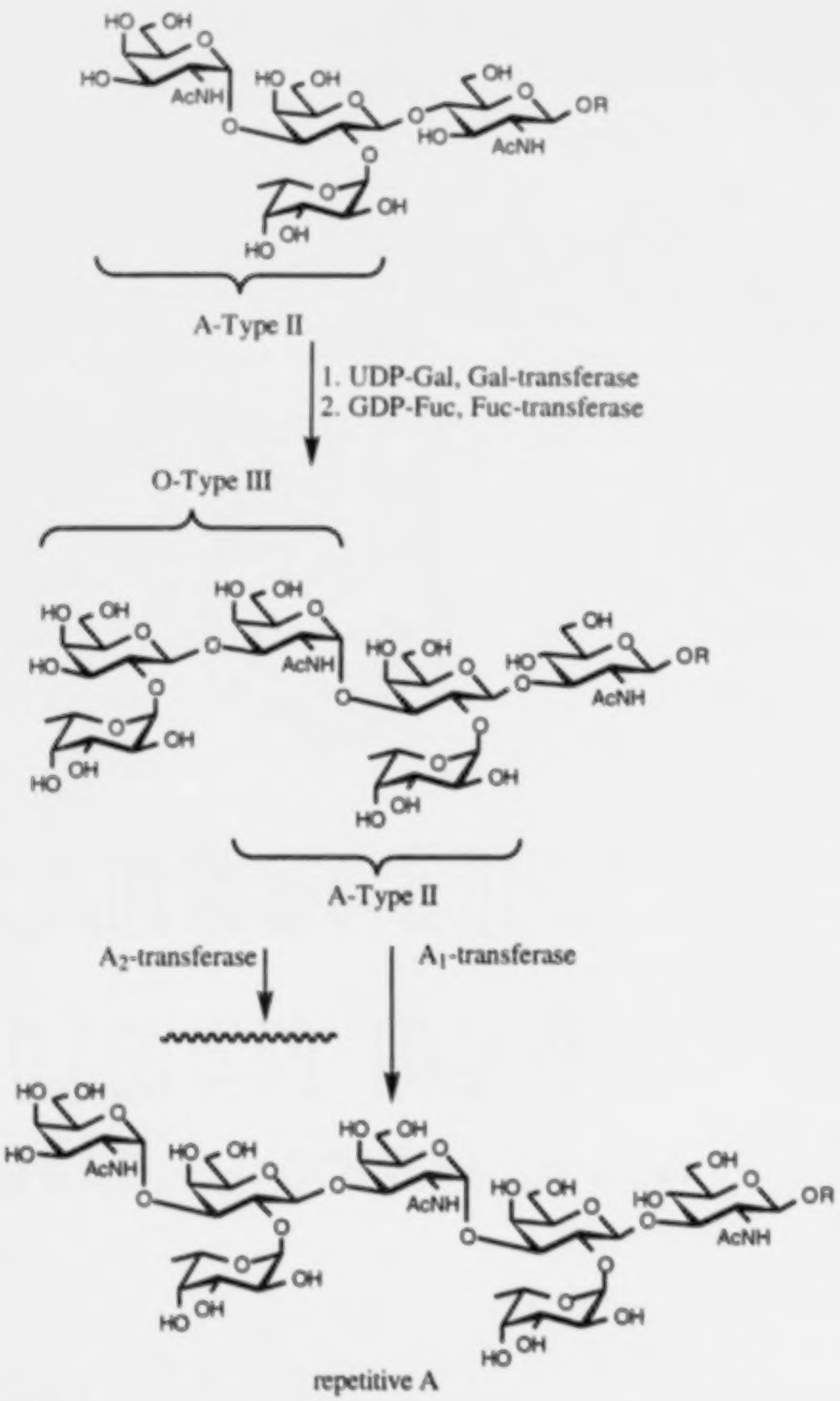

HO
OH
AcNH
OR
A-Type II
1. UDP-Gal, Gal-transferase
2. GDP-Fuc, Fuc-transferase
O-Type III
A-Type II
A$_2$-transferase
A$_1$-transferase
repetitive A

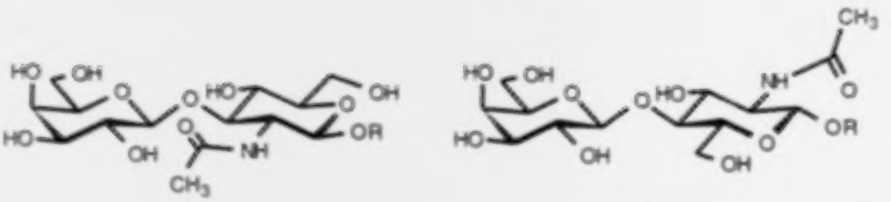
Fig 14. Proposed qualitative differences between A$_1$ and A$_2$ individuals.

The biosynthesis of these antigens is further complicated by the existence of two distinct fucosyltransferases which synthesize the O antigen. With regard to the occurrence of ABO antigens, individuals can be classified into one of four phenotypes[34, 76]. The first, and most common, are a group known as secretors. These individuals have the structures both on their erythrocytes and in their secretory tissues. Another group are those that have the antigens on their erythrocytes but not in their secretory tissues (non-secretors). Much less common are the "Bombay" and "para-Bombay" types, named for the city where individuals of this type were first discovered. Bombay individuals do not express the antigens in any tissue and para-Bombay types only in secretory tissues (Table 1). It was originally proposed that there were two genes involved, termed H and Se (for Secretor)[109]. The H-gene was responsible for the synthesis of the antigen and the Se-gene, a regulatory gene, regulated the activity of the H-gene. Therefore, in tissues where the Se gene was not present the ABO antigens would not be expressed in soluble form.

Table 1. Types of individuals based on expression of ABO blood group antigens.

INDIVIDUAL	Antigens on erythrocytes	Antigens in secretions
Secretor	+	+
Non-secretor	+	-
Bombay	-	-
para-Bombay	-	+

However, in the late 1970's, it was suggested that this model might not be correct. Up to that time it was believed that the H-enzyme would transfer fucose to both Type I and Type II chains. However, based on the comparison of hard-sphere molecular models of both substrates, Lemieux questioned whether the same enzyme could act on both molecules. Comparing the conformations of both Type I and Type II structures[110,111], he noted that the steric environment of the reactive hydroxyl group in these molecules is quite different (Fig 15). For Type I structures the C-5



TYPE I TYPE II

Fig 15. Conformation of Type I and Type II structures. Shown is the greater steric hindrance at Gal OH-2 in the case of the Type I acceptor due to the bulky acetamido group in GlcNAc.

hydroxymethyl group of the GlcNAc is close to the reactive Gal OH-2. However in the Type II structure, this hydroxyl group is in a much more sterically crowded environment, near the much larger acetamido group in GlcNAc. A short time later it was suggested that the enzymes were located in different tissues[112]. Eventually the two enzymes were separated and shown to occur in different tissues[113-117]. When tested with both Type I and Type II structures it was determined that the Se-enzyme preferred Type I structures while H-enzyme did not have such a rigid preference[115]. These results corroborate the tissue specificity mentioned above, that Type I chains are found mainly in secretory tissues and Type 2 chains are found primarily on erythrocytes.

The current model now accepted for expression of these antigens involves three, independent, though closely related, loci[118]. Two of them, the H/h, Se/se are two allele genes, with normal dominant and recessive patterns. The ABO locus is a three allele locus with both A and B dominant over O. Hence an individual who expresses A antigens on his erythrocytes, but not in secretory tissues has the following genotype: HH or Hh, sese, AA or AO. A "Bombay" individual has the genotype hh, sese, leaving them unable to synthesize the O antigen. In this case the ABO locus is irrelevant because without the H or Se enzymes to provide the O antigen, formation of the A or B antigens is not possible (Table 2).

Table 2. Possible genotypes of ABO individuals.

Pheno[1]	Locus	Secretor	Non-secretor	Bombay	para-Bombay
O	H/h	HH or Hh	HH or Hh	hh	hh
	Se/se	SeSe or Sese	sese	sese	SeSe or sese
	ABO	OO	OO	N/A[2]	OO
A	H/h	HH or Hh	HH or Hh	hh	hh
	Se/se	SeSe or Sese	sese	sese	SeSe or sese
	ABO	AA or AO	AA or AO	N/A	AA or AO
B	H/h	HH or Hh	HH or Hh	hh	hh
	Se/se	SeSe or Sese	sese	sese	SeSe or sese
	ABO	BB or BO	BB or BO	N/A	BB or BO
AB	H/h	HH or Hh	HH or Hh	hh	hh
	Se/se	SeSe or Sese	sese	sese	SeSe or sese
	ABO	AB	AB	N/A	AB

[1] Phenotype of individual

[2] Not applicable. Bombay individuals are incapable of producing the O antigen and thus cannot make A or B antigens.

1.6 CLONING OF A, B AND H TRANSFERASES

In 1990 the A, B and H transferases were cloned[119-122]. To date, the enzyme resulting from the expression of the Se gene has not been cloned. As well, the enzyme from a cis-AB individual has not been cloned.

1. 6. 1 CLONING OF THE A AND B TRANSFERASES

The A and B tranferases were cloned by Yamamoto et. al. using four cDNA libraries constructed from four different human colon adenocarcinoma cell lines[119,121]. As a probe to tag the correct DNA, a portion of purified RNA from lung tissue was used[123]. From these studies it was determined that the number of amino acids in both the A and B-transferases are identical and that the amino acid sequences differ at only four residues. The sequences of both are shown in Fig. 16. The changes (from the A-transferase to the B-transferase) are: Arg^{176}→Gly, Gly^{235}→Ser, Leu^{266}→Met, Gly^{268}→Ala. All these substitutions occur near the carboxy terminus of the protein, the region known to be the catalytic portion of both enzymes[47,124].

```
A-transferase: M A E V L R T L A G K P K C H A L R P M I L F L I M L V L V
B-transferase:

A            : L F G Y G V L S P R S L M P G S L E R G F C M A V R E P D
B            :

A            : H L Q R V S L P R M V Y P Q P K V L P C R K D V L V V T P
B            :

A            : W L A P I V W E G T F N I D I L N E Q F R L Q N T T I G L T
B            :

A            : V F A I K K Y V A F L K L F L E T A E K H F M V G H R V H
B            :

A            : Y Y V F T D Q P A A V P R V T L G T G R Q L S V L E V R A
B            :                                                       G

A            : Y K R W Q D V S M R R M E M I S D F C E R R F L S E V D Y
B            :

A            : L V C V D V D M E F R D H V G V E I L T P L F G T L H P G F
B            :                                                         S

A            : Y G S S R E A F T Y E R R P Q S Q A Y I P K D E G D F Y Y L
B            :                                                           M

A            : G G F F G G S V Q E V Q R L T R A C H Q A M M V D Q A N
B            :   A

A            : G I E A V W H H D E S H L N K Y L L R H K P T K V L S P E
B            :

A            : Y L W D Q Q L L G W P A V L R K L R F T A V P K N H Q A V
B            :

A            : R N P
B            :
```

Fig 16. Amino Acid sequences of the A and B-transferases. Only the differences in the B-transferase are shown, and are highlighted in bold. The proposed membrane anchoring region is underlined. One site of potential N-glycosylation is double underlined.

Table 3. Synthetic enzymes (chimeras) produced to investigate influence of amino acid substitution in A and B transferases.

CHIMERA	AA^{176}[1]	AA^{235}	AA^{266}	AA^{268}	Phenotype
AAAA	Arg	Gly	Leu	Gly	A
AAAB	Arg	Gly	Leu	Ala	A
AABA	Arg	Gly	Met	Gly	AB
AABB	Arg	Gly	Met	Ala	B
ABAA	Arg	Ser	Leu	Gly	A
ABAB	Arg	Ser	Leu	Ala	A (B)[2]
ABBA	Arg	Ser	Met	Gly	AB
ABBB	Arg	Ser	Met	Ala	B
BAAA	Gly	Gly	Leu	Gly	A
BAAB	Gly	Gly	Leu	Ala	A
BABA	Gly	Gly	Met	Gly	AB
BABB	Gly	Gly	Met	Ala	B
BBAA	Gly	Ser	Leu	Gly	A
BBAB	Gly	Ser	Leu	Ala	A (B)[2]
BBBA	Gly	Ser	Met	Gly	AB
BBBB	Gly	Ser	Met	Ala	B

[1] Amino Acid at position indicated
[2] Showed weak B transferase activity

In related experiments[120], Hakomori and coworkers have also made proteins with all 16 possible permutations (chimeras) of the variable amino acids (Table 3). Of the 16 chimeras, four have both A and B-transferase activity and hence one of them might correspond to the enzyme found in cis-AB individuals. From these studies the authors concluded that the first (Arg→Gly) substitution was not important in determining the specificity of the sugar nucleotide accepted. In all cases the third (Leu→Met) and fourth (Gly→Ala) substitutions were the most critical. For example, those proteins with amino acids corresponding to the A-transferases (denoted as AA) in the third and fourth positions expressed A-transferase activity regardless of what amino acids were in the first two positions. A similar pattern was observed for proteins that carried the BB substitution at positions three and four. The second substitution (Gly→Ser) is important only when the sequence at the third and fourth substitution is AB. Chimeras with the BAB at these positions showed A activity along with weak B activity. Those that were AAB showed only A activity. Finally, the protein exhibited strong AB activity only when the substitution at positions three and four was BA.

Using computer modeling studies, the last two substitutions in the B transferase were shown to decrease the flexibility in the protein around these amino acids. The authors proposed that the size difference between the galactose and N-acetylgalactosamine, in combination with reduced enzyme flexibility might be of critical importance in determining sugar nucleotide specificity. It should be noted, however, that the location of the active site for either enzyme is not known.

These studies also determined that the product of the O gene is a non-functional protein, with the nucleic acid base sequence identical to the A-transferase, except for a single base deletion. This deletion, of a guanosine at position 258, results in a shifting of the reading frame during protein synthesis, producing the inactive protein.

1. 6. 2 CLONING OF THE H -TRANSFERASE

The gene which encodes for the H-fucosyltransferase which produces the O antigen has also been cloned and expressed[122]. Using isolated human DNA segments, Lowe and associates cloned an enzyme which was kinetically similar to the human H-enzyme. It was also shown that the cloned enzyme was distinct from the Se-enzyme. Fig 17 shows the amino acid sequence of the enzyme which is very similar in length to the A and B-transferases.

1. 6. 3 MORPHOLOGY

All three enzymes are morphologically similar to other known glycosyltransferases[47, 124]. They all possess an amino-terminal cytoplasmic tail, a transmembrane domain (shown in Figs. 16 and 17), and a carboxy-terminal catalytic region located inside the Golgi Apparatus. It is believed that these enzymes are located primarily in the *trans*-Golgi[125, 126]. Table 4 shows a comparison of the three

MW LRSHRQLCLAFLLVCVLSVIFFLHIHQD

SFPHGLGLSILCPDRRLVTPPVAIFCLPGT

AMGPNASSSCPQHPASLSGTWTVYPNGRF

GNQMGQYATLLALAQLNGRRAFILPAMHA

ALAPVFRITLPVLAPEVDSRTPWRELQLHD

WMSEEYADLRDPFLKLSGFPSWTFFHHLR

EQI RRQEFTLHDHLREEAQSVLGQLRLGR

TGDRPRTFVGVHVRRGDYLQVMPQRWKV

VGDSAYLRQAMDWFRARHEAPVFVVTSNG

MEW CKENIDTSQGDVTFAGDGQEATPWK

DFALLTQCNTIMTIGTFGFWAAYLAGGDT

VYLANFTQLPDSEFLKIFKPEAAFLPEWVG

INADLSPLWTLAKP

Fig 17. Amino Acid sequence of the H-transferase. The proposed membrane anchoring region is underlined. Sites of potential N-glycosylation are double underlined.

Table 4. Comparison of morphology of A, B and H transferases.

	Number of amino acids in each domain			
Enzyme	Amino Terminal	Trans-membrane	Carboxy-Terminal	Chromosome Location
A	16	21	316	9
B	16	21	316	9
H	8	17	340	19

enzymes and the number of amino acids present in the three regions. Additionally, it has been determined that the A and B-transferases are located on chromosome 9 while the H-fucosyltransferase is located on chromosome 19.

The sites of potential N-glycosylation are shown in Figures 16 and 17. The A and B-transferase have only one site, while the H-transferase has two sites. A recent report has identified that the A-transferase binds an antibody specific for the A-antigen, indicating the presence of this structure on the enzyme[127]. Furthermore, treatment with N-glycanase, an enzyme known to remove only oligosaccharides attached to asparagine, resulted in loss of binding by the same antigen. Therefore this A-antigen is located at the non-reducing end of an N-linked oligosaccharide. Other information concerning glycosylation of these proteins is not known.

1. 7 SUBSTRATE SPECIFICITY OF A AND B TRANSFERASES

1. 7. 1 DONOR SPECIFICITY

Under normal physiological conditions, the enzymes transfer their sugar nucleotide donors with rigid specificity. However, it has been shown that *in vitro*, the B-transferase will use UDP-GalNAc and transfer it to the O antigen thus forming an A antigen[128,129]. The converse reaction, the transfer of Gal from UDP-Gal to the O antigen catalyzed by the A-transferase, has also been reported[130]. Thus under unnatural conditions, each enzyme is capable of cross reacting with the sugar-nucelotide normally used by the other. It has also been shown that a structurally related bovine B-transferase will transfer glucose from UDP-Glc[131].

Of greater importance is the observation that certain B individuals can express small amounts of the A antigen on their cells[132]. These individuals possess a very active B-transferase (suggested to be greater than 5 times the normal activity) which appears to transfer small amounts of UDP-GalNAc. This could be important in cancer patients who possess increased levels of B-transferase, and may also provide an explanation for the observed expression of foreign blood group antigens in cancerous tissue[76].

Also of interest is the report that, after concentration of large volumes of O serum, an enzyme was obtained that could synthesize the A antigen[133]. As above, the authors postulated that the presence of the this A transferase could result in the aberrant expression of A-antigens in O individuals in some carcinomas.

In the early 1970's, it was discovered[134] and then later confirmed[135] that UDP-Gal acts as a competitive inhibitor of the A-transferase. Hence, the A-transferase appears to bind the sterically less demanding UDP-Gal, but turns over the product at a much slower rate. As well, UDP inhibits both the A and B-transferases[134]. This inhibition follows a common pattern for the glycosyltransferases, which are known to be inhibited by their free NDP moieties. It is not known whether the enzymes will recognize other sugar nucleotides, either naturally occurring or synthetic (i.e deoxygenated or fluorinated derivatives).

1. 7. 2 ACCEPTOR SPECIFICITY

Until this investigation, a detailed examination of the acceptor substrate specificity of these enzymes had not been carried out. The information that was known had been obtained using naturally occurring substrates. From these substrates it was determined that both enzymes appear to recognize all glycoconjugates possessing a terminal O disaccharide antigen of α-L-Fuc-(1→2)-β-D-Gal*p*-OR (Fig 12).

1. 8 SUBSTRATE SPECIFICITY OF H AND Se TRANSFERASES

1. 8. 1 DONOR SPECIFICITY

No studies have been carried out concerning the donor specificity of these fucosyltransferases. While deoxygenated analogs of GDP-fucose have been tested as substrates for another fucosyltransferase[136], they have not been tested with these enzymes. Inhibition studies[137] of an α (1→2) fucosyltransferase from rat intestine with a variety of GDP analogs have been carried out and it was shown that the enzyme is inhibited by not only GDP, but also GMP, GTP, and GDP-Man.

1. 8. 2 ACCEPTOR SPECIFICITY

The acceptor specificity of these enzymes has been briefly mentioned above. The Se enzyme prefers Type I structures, but also recognizes Type III acceptors. The H enzyme appears to be less strict, recognizing both Type I and Type II acceptors. The H gene also shows a higher affinity for β-Gal-O-phenyl, the minimum carbohydrate unit required for recognition. It has also been shown that the 6-deoxy derivative of galactose can act as a substrate[138]. No other studies using unnatural substrates have appeared.

1.9 COMMON CARBOHYDRATE-PROTEIN INTERACTIONS

Prior to starting this work, a survey was made of the literature to determine what common motifs had been reported for the ways in which proteins recognize carbohydrates. Such information would be useful in determining what substrate analogs to prepare as potential inhibitors for these enzymes. In particular, since all four enzymes (A, B, H and Se) recognize substrates containing galactose, and transfer to this residue, special attention was given to galactose recognizing proteins.

Carbohydrate-protein interactions can be divided into two main classes: hydrogen bonding interactions and van der Waals stacking interactions[139]. Given the polyhydroxylated structure of carbohydrates, the first type might appear to be the most important. However, it has been shown that, frequently, usually only one or two of the four possible hydroxyl groups on any given monosaccharide residue are critical for binding. That is, these hydroxyl groups are involved in an interaction such that removal of the hydroxyl group completely destroys the ability of the substrate to bind. Water molecules also contribute to hydrogen bonding interactions. In many crystal structures, water molecules are involved in essential hydrogen bonding networks between the carbohydrate and the protein. In some cases water molecules have been found buried deeply in the binding pocket[140].

Van der Waals stacking interactions result from the sandwiching of sugar residues between aromatic amino acids, such as tryptophan, tyrosine, phenylalanine and in at least one case, histidine[141]. Tryptophan is the most common amino acid involved in stacking interactions. It has been suggested that these hydrophobic interactions dictate much of the substrate specificity of the protein. This is achieved by preventing the binding of epimeric structures of sugars either due to steric hindrance, or by creating an unfavorable polar environment by placement of a polar hydroxyl group too close to a hydrophobic amino acid side chain.

1. 10 SPECIFIC INTERACTIONS IN GALACTOSE RECOGNIZING PROTEINS

1. 10. 1 WORK OF LEMIEUX

Over the past decade, Lemieux and associates have published many thorough studies looking at the binding of a number of galactose-containing blood-group oligosaccharides to plant lectins. As well, Quiocho and coworkers have reported a number of crystallographic studies on proteins which bind and transport galactose, and related structures in bacteria. Two other studies have also been published recently both using lactose, a galactose containing disaccharide, as the bound carbohydrate unit. One study investigated the complex between lactose and a bacterial protein, while the other dealt with a plant lectin crystallized with this disaccharide.

The work of Lemieux[142-144] has concentrated primarily on the recognition of the Lewis b and Y blood group determinants respectively, α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc, and α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc (Fig 18). Both structures bind to a plant lectin from *Griffonia simplicifolia* (GS4). A recent paper[143] reported the crystal structure of this lectin with the tetrasaccharide Lewis b antigen. In that structure, the

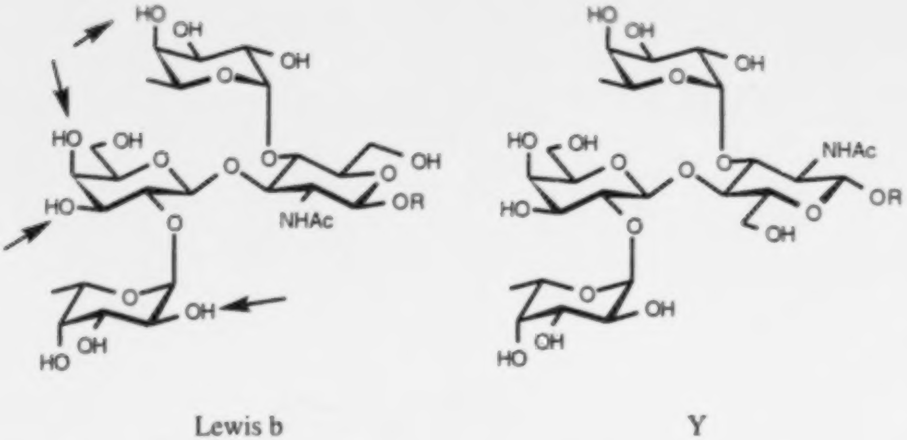


Fig 18. Comparison of the Lewis b and Y antigens. Both are bound by the GS4 lectin. Hydroxyl groups in Lewis b involved in key polar interactions are shown with arrows.

galactosyl residue was shown to contain two of the four hydroxyl groups critical for binding. These crucial hydroxyl groups, are involved in what Lemieux terms "key polar interactions" with the protein[177]. The four hydroxyl groups involved in these key polar interactions are Gal 3-OH and Gal 4-OH, the 4-OH of the α-(1→4) linked fucose and the 2-OH of the α-(1→2) linked fucose (Fig 18). The galactose is buried deep in the binding pocket and the 3,4 diol donates hydrogen bonds to an aspartate residue (Asp^{89}) at the bottom of the binding pocket through a bidentate arrangement

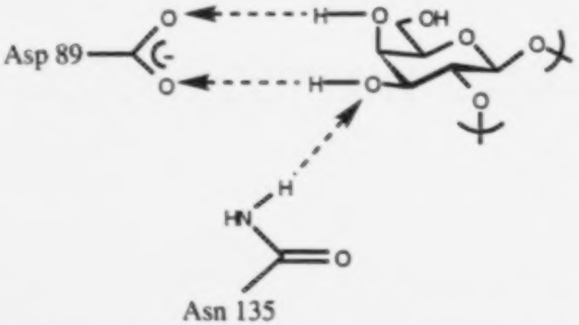


Fig 19. Important hydrogen bonding interactions between the galactosyl residue of Lewis b and GS4. Hydrogen bonds are drawn as H→acceptor throughout.

(Fig. 19, Hydrogen bonds are shown throughout the text as H→acceptor). As well, the O-3 acts as a hydrogen bond acceptor with the side chain amide of Asn^{135}. Five aromatic amino acids (Trp^{133}, Trp^{138}, Tyr^{105}, Tyr^{223}, and Phe^{108}) also are present in the binding pocket and make van der Waals contacts with the carbohydrate epitope.

1. 10. 2 WORK OF QUIOCHO

Quiocho's work in the area[145-150] has centered on two binding proteins which can recognize galactose related structures. One of these, the arabinose-binding protein (ABP), recognizes L-arabinose as its natural substrate. Another protein, the D-galactose/D-glucose binding protein (GBP), can recognize either monosaccharide.

Very elegant and detailed studies with crystals of ABP have been carried out [145, 147, 150]. It is known that the protein recognizes both galactose (Gal) and D-fucose (6-deoxy-D-galactose, D-Fuc) in addition to L-arabinose (Ara) as substrates. L-arabinose and D-galactose have the same configuration at their secondary hydroxyl groups, but in the former a hydrogen replaces the hydroxymethyl group at C-5. (Fig 20) Both Gal and Ara are excellent substrates possessing approximately the same binding ability[147]. D-Fuc binds more weakly, with a dissociation constant about an order of magnitude higher[147]. A paper published after this thesis work began reported that ABP also binds 6-fluoro-galactose[150]. Interestingly, although the fluoro-substituted carbohydrate is intermediate in steric bulk between D-Gal and D-Fuc, this derivative actually binds more weakly than any of the other derivatives.

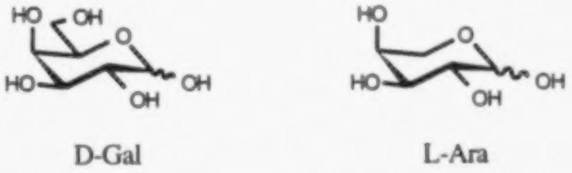


Fig 20. Comparison of D-galactose structure with L-arabinose.

As can be seen from a schematic diagram of the important hydrogen bonding interactions with L-Ara (Fig 21)[145], the binding pocket contains amino acids with side chains containing derivatives of carboxylic acids. Most of the important interactions are between the carbohydrate and four carboxylates, two asparagines, and the guanidinium side chain of arginine. As well three water molecules are involved in the hydrogen bonding network. This extensive hydrogen bonding network is common to all crystal structures of the previously mentioned arabinose derivatives. Note that similar to the aforementioned work of Lemieux, an asparagine residue forms bidentate hydrogen bonds with the Gal OH-3 and OH-4. An aspartate is used so that the enzyme can recognize both the α and β-anomers of arabinose.

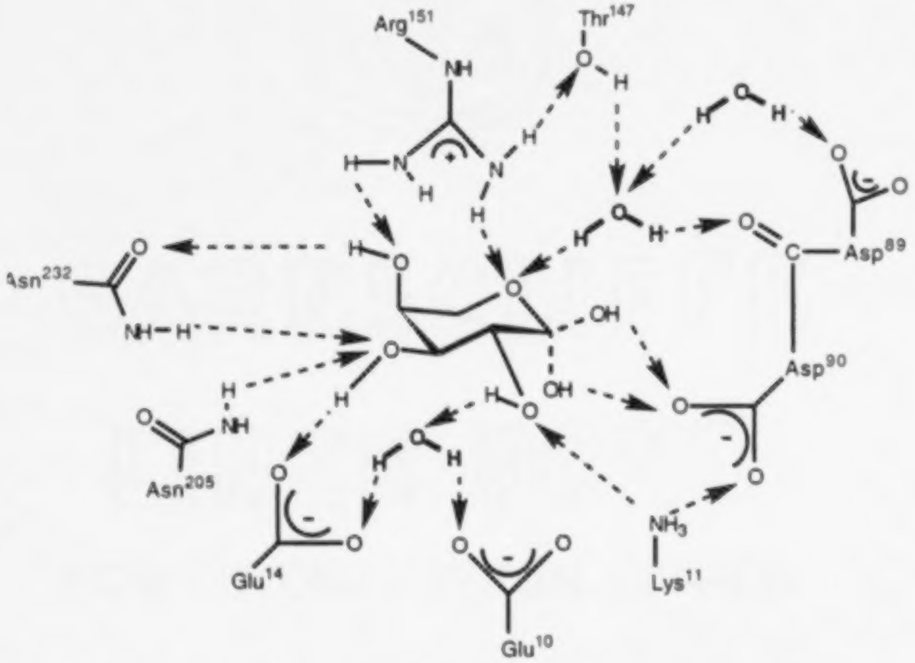


Fig 21. Important interactions in the binding pocket between ABP and L-arabinose, showing the extensive hydrogen bonding between the carbohydrate and protein. Three water molecules are included in the hydrogen bonding network. The binding site consists of a number of carboxylates, as well as two other acyl-derived amino acid side chains, arginine and asparagine.

The pattern of binding of the derivatives modified at the 6 position was attributed to a combination of the shifting of water molecules, polar effects and the electrostatic repulsion of Asp[89] by the electronegative fluorine[147,150]. When binding its natural substrate, Ara, the protein has two water molecules bound in the area of the ring oxygen (Fig 22 A)[147].

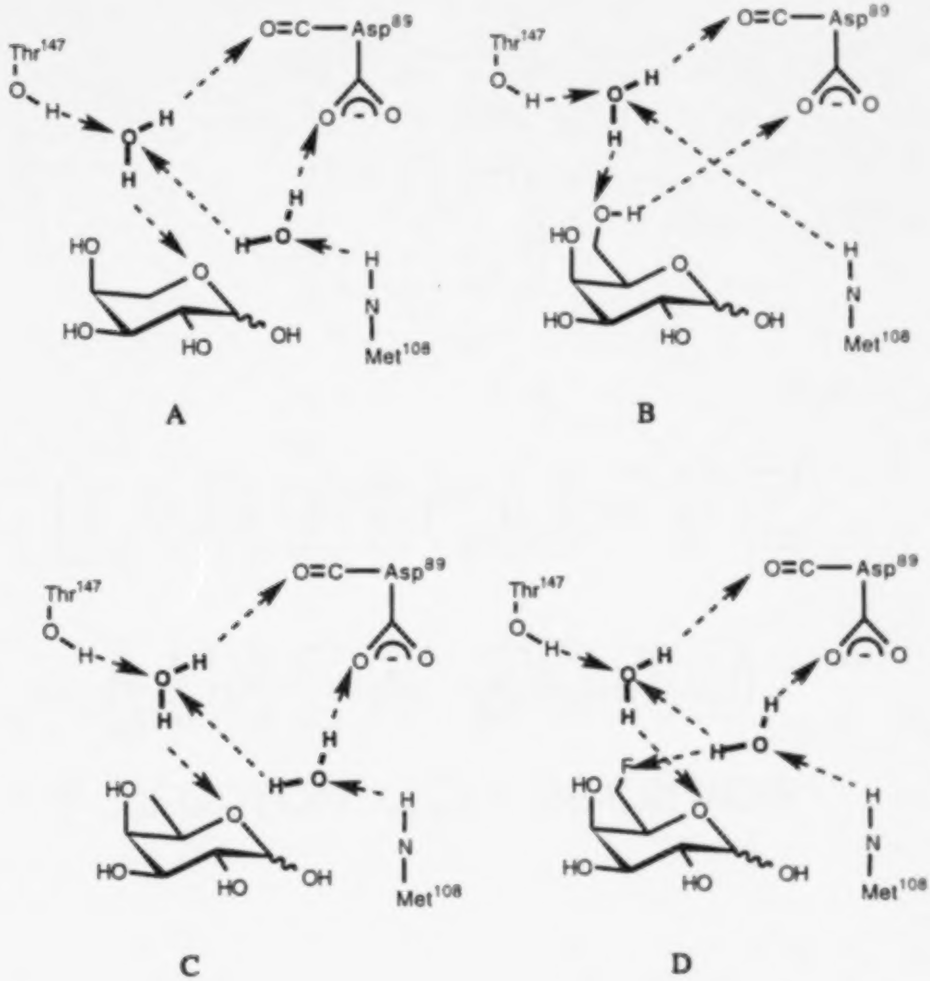


Fig 22. Comparison of hydrogen bonding interactions in the area of C-5 in crystal structure of ABP with L-Ara, D-Gal, D-Fuc and 6F-D-Gal.

When binding the D-Gal derivative[147] (Fig 22 B), the binding site is too crowded with both water molecules, and thus one of the water molecules is expelled to bulk solvent. The hydroxymethyl group at C-5 replaces one of the critical binding interactions with the protein previously formed between a water molecule and the aspartate residue (Asp^{89}).

With the less bulky D-Fuc derivative[147] (Fig 22 C), two water molecules are again incorporated in the binding pocket. The hydrogen bonding arrangement is identical to those formed when the protein binds Ara, but now the hydrophobic character of the C-6 methyl group destabilizes the complex by introducing a nonpolar group into a polar region.

Introducing the strongly electronegative fluorine in 6-fluoro-galactose into the active site causes a repulsion between the negatively charged Asp^{89} and the C-6 fluorine[150]. As a result, the aspartate rotates its position, allowing the incorporation of another water molecule into the complex (Fig 22 D). This second water molecule donates a hydrogen bond to the fluorine as well as Asp^{89}. So while the fluorinated derivative can form more hydrogen bonds than the D-Fuc derivative, the stabilization achieved by the formation of these hydrogen bonds is overcome by the strong destablization caused by the electrostatic repulsion of the fluorine and Asp^{89}.

In 1990, Martin et al. reported another similar repulsion in a complex between the enzyme phosphorylase and 2-fluoro-α-D-glucose-1-phosphate[151]. These results with fluorinated carbohydrate analogs cast into doubt the presently accepted dogma[152, 153] that substitution of a hydroxyl group with a fluorine probes only the hydrogen bonding energetics.

Quiocho's studies using GBP have also uncovered that an aspartate residue plays an important role in the binding of both galactose and glucose to the protein[146]. In this particular case, however, only one of the aspartate oxygens hydrogen bonds to the 4-OH group, depending upon what substrate is being bound (Fig 23). Another aspartate residue is also important in forming hydrogen bonding interactions with the hydroxyl group at C-2 and C-3. GBP can bind either anomer of the substrate, using, as does **ABP**, a resonance-stabilized charged amino acid. While ABP uses an aspartate to carry out this function, GBP uses an arginine.

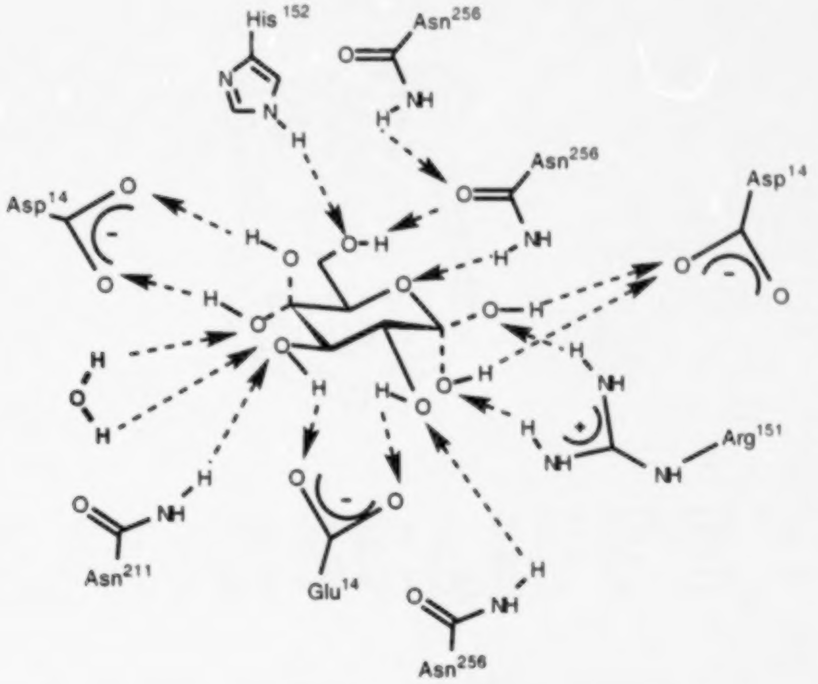


Fig 23. Schematic diagram of the important binding interactions in the recognition of Gal or Glc by GBP.

Another similarity between the two enzymes is the presence of important hydrophobic interactions with aromatic amino acids. Both Ara and Gal, especially as their β-anomers, provide a hydrophobic surface on the bottom of the molecule that lends itself well to van der Waals stacking interactions.

1. 10. 3 WORK OF BERGHUIS

The recently published crystal structure of lactose bound to a bacterial protein from *E. coli.* again points to the presence of important carboxylic acid derivatives in the active site[154]. The protein recognizes the disaccharide mainly though the galactose residue. In this case, while a glutamate residue accepts a hydrogen bond from OH-4, most of the interactions are with the side chain amide groups of asparagine and glutamine. However, carboxylates and amino groups also help to stabilize the structure. The hydroxyl groups at C-3 and C-2 are involved in bidentate hydrogen bonding to an asparagine residue, while OH-6 participates in an extensive hydrogen bond network with two of the four water molecules sequestered in the protein. As in other crystal structures, van der Waals interactions between the sugar and a tryptophan residue are present (Fig 24 A).

1. 10. 4 WORK OF SHARON

Finally, lactose also crystallizes with a lectin from *Erythrina corallodendrun*, and was the subject of a recent report by Sharon[155]. As with the bacterial protein, this lectin uses only the Gal moiety of the disaccharide for binding. The 3,4 diol is involved in the hydrogen bonding network, binding to an aspartate residue in a matter identical to Lemieux's GS4 structure. The 3-OH is also hydrogen bonded to an asparagine and to glycine via an NH in the peptide backbone. In addition to the hydrogen bond to aspartate, OH-4 also hydrogen bonds to the backbone NH in an alanine residue. The other important hydrogen bonds are between the side chain of asparagine and the 6 hydroxyl group and the inclusion of a water molecule, via a hydrogen bond to the aspartate. The lectin requires calcium for binding and the water molecule helps to bind this metal ion. Finally, a phenylalanine residue (not shown), located on the bottom side of the Gal-ring, stabilizes the structure through van der Waals interactions (Fig. 24 B).

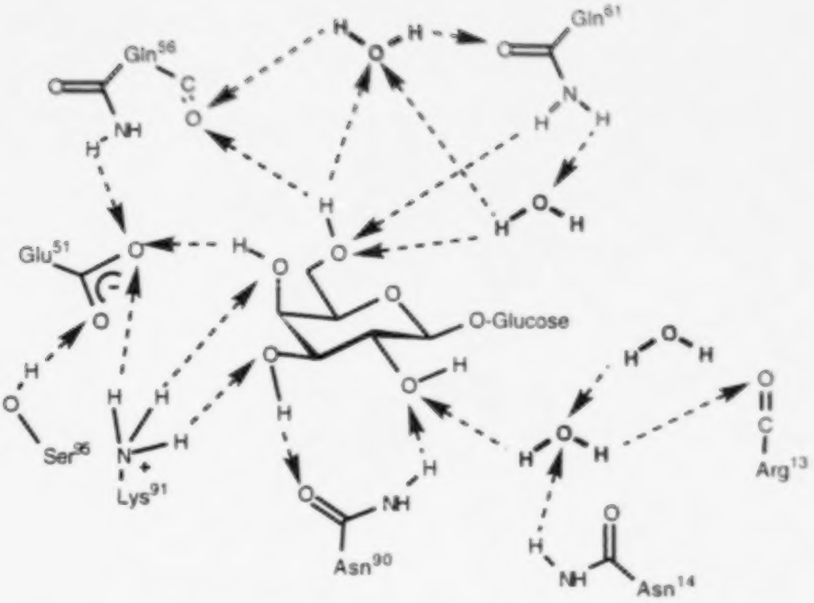

Gln^{56}
Gln^{61}
Glu^{51}
Ser^{95}
Lys^{91}
O-Glucose
Arg^{13}
Asn^{90}
Asn^{14}

Fig 24 A. Schematic representation of lactose binding to heat-labile enterotoxin from *E.coli.* Water molecules important in binding are highlighted in bold.

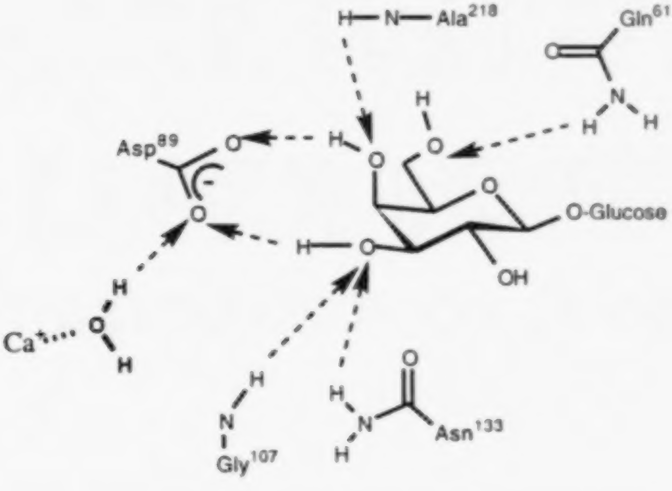

H—N—Ala^{218}
Gln^{61}
Asp^{89}
O-Glucose
OH
Ca
Gly^{107}
Asn^{133}

Fig 24 B. Schematic representation of lactose binding to the plant lectin *Erythrina corallodendron.*

It appears, therefore, that a common structural motif for carbohydrate-protein interactions is the presence of a number of carboxylate, amide or guanidinium groups in the active site, as well as sandwiching by hydrophobic aromatic amino acids. However, a caveat should be inserted here cautioning that these proteins are very different from enzymes, in that their function is to bind carbohydrates and transport them, not to bind them and than carry out a chemical reaction. It is not unreasonable to anticipate then, that such extensive hydrogen bonding interactions might not be present in enzymes due to the need for them to readily expel their reaction product. Nevertheless, these crystal structures provide useful information, at least as a starting point, to hypothesize what groups might be present in the active site.

1. 11 SCOPE OF PROJECT

As mentioned above, before this investigation little information was available on the substrate specificity of these glycosyltransferases. This thesis research involved first the preparation of a number of systematically modified analogs of the natural enzyme acceptor substrates. These analogs were then tested as potential substrates or inhibitors. For those compounds showing activity as either an acceptor or inhibitor, the appropriate kinetic constants were determined.

At the outset, we realized it was unlikely that an extremely potent inhibitor would be found. However, it was anticipated that this type of study would provide crucial information regarding important carbohydrate-protein interactions in the active sites of these enzymes. This information would greatly facilitate rational inhibitor design in the future.

The minimum structure recognized by the H enzyme is the monosaccharide β-D-Gal*p*-OR[76]. The A and B transferases in turn recognize the H disaccharide, α-L-Fuc*p*-(1→2)-β-D-Gal*p*-OR, as a minimum acceptor[76]. In the present work we chose R to be octyl (**1**, **2** , Fig 26) because the presence of this hydrophobic aglycon would simplify the enzymatic assays by allowing the use of reverse phase (C_{18}) cartridges to separate and quantitate the product[156]. We concentrated our efforts on the galactosyl moiety of the H disaccharide, making a number of analogs of this structure. We chose the synthetic strategy such that the modified galactose residue was prepared first. This approach allowed us to take a portion of these products and use them for the fucosyltransferase studies. To the remainder of the sample, fucose could then be added, providing the disaccharide analogs required for the A and B-transferase studies (Fig 25).

From the crystal structure data presented above, we believed it was likely that the active site would contain at least one carboxylate residue. Due to the close structural similarity between the Lewis b antigen used in Lemieux's work, and the disaccharide required for A and B transferase activity, we hypothesized that the 3 and 4 hydroxyl groups would be involved in an interaction with the carboxylate. This hypothesis was especially attractive in that the mechanism we proposed for transfer involved a base in the active site. Thus, this proposed carboxylate would be critical in both substrate recognition and catalysis. Keeping this in mind, we chose to prepare eighteen structural analogs of both the monosaccharide and disaccharide described above.

The oligosaccharide analogs chosen (Fig 26) were those where the hydroxyl groups at the 3, 4, and 6 positions in the galactosyl residue were replaced, independently, with H (**3-5, 21-23**), F (**6-8, 24-26**), O-methyl (**9-11, 27-29**), NH_2 (**14-16, 32-34**) and NHAc (**17-19, 35-37**). These modifications can be divided into two groups, those modifications that probe hydrogen bonding interactions and those that probe steric interactions.

1. 11. 1 MODIFICATIONS PROBING HYDROGEN BONDING INTERACTIONS

Substitutions of hydroxyl groups by either H or F are sterically conservative modifications and can thus provide insights into whether the hydroxyl group removed

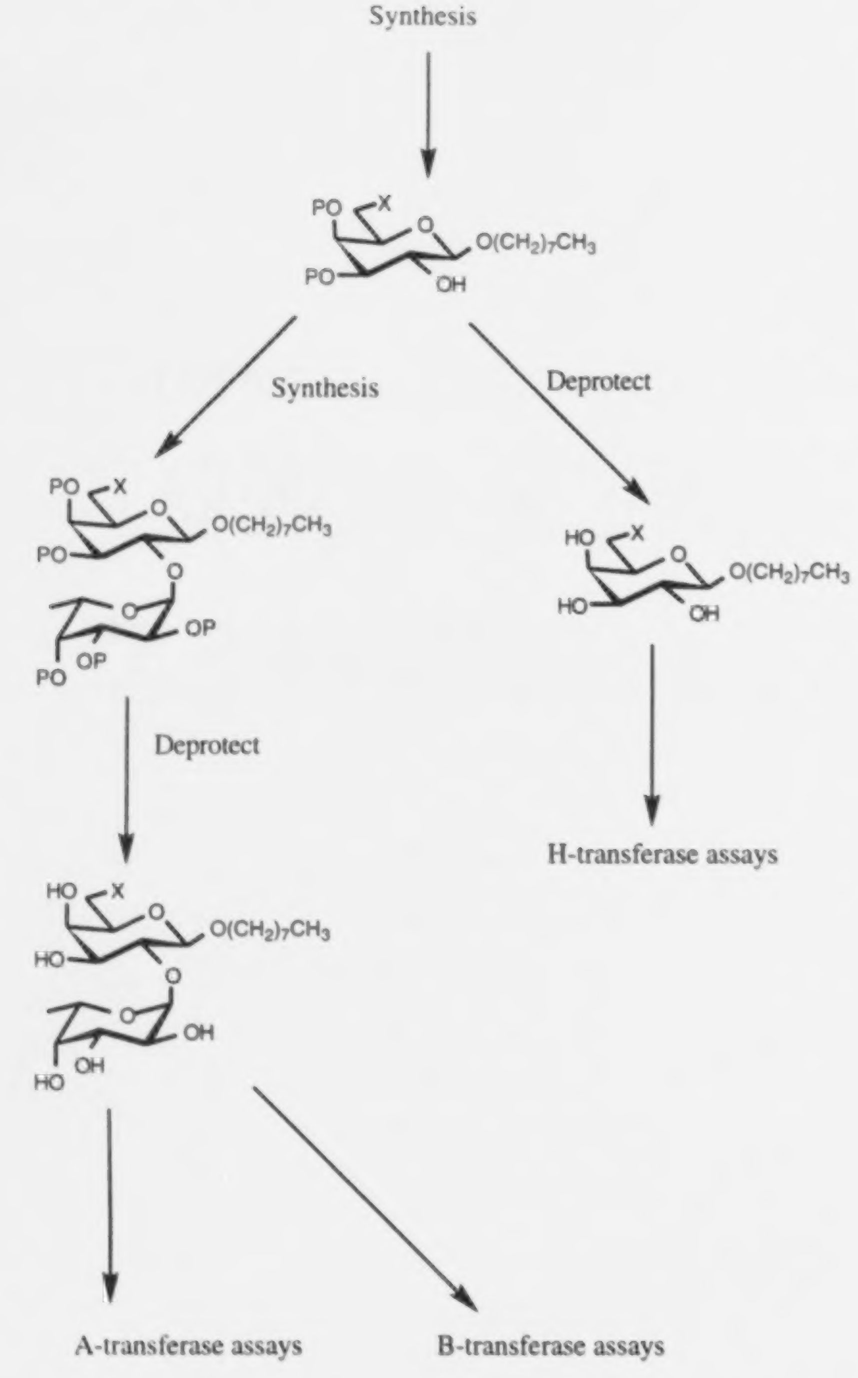
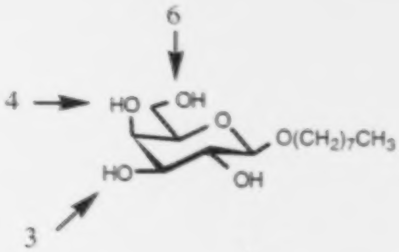
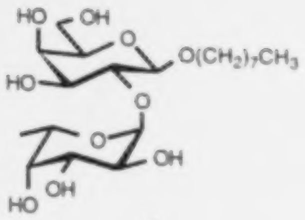

Synthesis
PO
X
O
O(CH$_2$)$_7$CH$_3$
PO
OH
Synthesis
Deprotect
PO
X
O
O(CH$_2$)$_7$CH$_3$
PO
O
O
OP
PO
OP
HO
X
O
O(CH$_2$)$_7$CH$_3$
HO
OH
Deprotect
HO
X
O
O(CH$_2$)$_7$CH$_3$
HO
O
O
OH
HO
OH
H-transferase assays
A-transferase assays
B-transferase assays

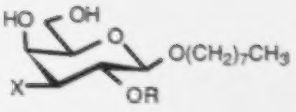
Fig 25. Outline of thesis strategy, shown here for a Gal OH-6 modified analog. X = modification, P = protecting group.


6
4
3
HO
OH
O
O(CH₂)₇CH₃
HO
OH

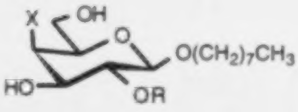
1


HO
OH
O
O(CH₂)₇CH₃
HO
O
O
OH
HO
OH

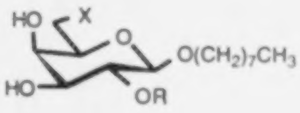
2


HO
OH
O
O(CH₂)₇CH₃
X
OR

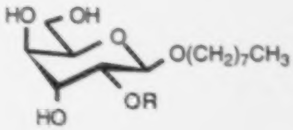
3 R = α-Fuc, X = H
6 R = α-Fuc, X = F
9 R = α-Fuc, X =OCH_3
14 R = α-Fuc, X = NH_2
17 R = α-Fuc, X = NHAc
21 R = H, X = H
24 R = H, X = F
27 R = H, X = OCH_3
32 R = H, X = NH_2
35 R = H, X = NHAc


X
OH
O
O(CH₂)₇CH₃
HO
OR

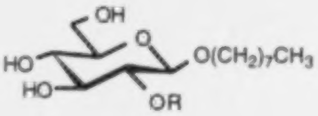
4 R = α-Fuc, X = H
7 R = α-Fuc, X = F
10 R = α-Fuc, X =OCH_3
15 R = α-Fuc, X = NH_2
18 R = α-Fuc, X = NHAc
22 R = H, X = H
25 R = H, X = F
28 R = H, X = OCH_3
33 R = H, X = NH_2
36 R = H, X = NHAc


HO
X
O
O(CH₂)₇CH₃
HO
OR

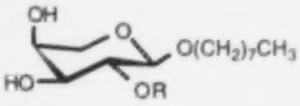
5 R = α-Fuc, X = H
8 R = α-Fuc, X = F
11 R = α-Fuc, X =OCH_3
16 R = α-Fuc, X = NH_2
19 R = α-Fuc, X = NHAc
23 R = H, X = H
26 R = H, X = F
29 R = H, X = OCH_3
34 R = H, X = NH_2
37 R = H, X = NHAc


HO
OH
O
O(CH₂)₇CH₃
HO
OR

12 R = α-Fuc
30 R = H


OH
O
O(CH₂)₇CH₃
HO
HO
OR

13 R = α-Fuc
31 R = H


OH
O
O(CH₂)₇CH₃
HO
OR

20 R = α-Fuc
38 R = H

Fig 26. Substrates and target molecules.

was involved in a critical hydrogen bond interaction with the protein combining site[150, 153]. Deoxygenation provides information about whether that hydroxyl group is acting either as an acceptor or donor with the enzyme. At the time that this work was begun, it was believed that substitution with fluorine, which can act as a hydrogen-bond acceptor but not a hydrogen-bond donor, would give insight into whether the hydroxyl group was acting as a hydrogen-bond acceptor. However, as mentioned previously, during the course of this work, a paper by Quiocho appeared casting doubt onto that assumption[150]. This aspect will be discussed further in Chapter 2 in conjunction with the biochemical results.

The amino compounds were synthesized to probe for the existence of a negatively charged amino acid residue in the active site. At physiological pH, the amine would be protonated, and therefore it was expected that if there were a negatively charged group near the positively charged amine, a strong ionic interaction might result. Such a compound could serve as a potent inhibitor of the enzyme via a tightly held enzyme-substrate complex.

1. 11. 2 MODIFICATIONS PROBING STERIC INTERACTIONS

Methylation of a hydroxyl group probes not only the hydrogen bonding requirements of the enzyme, but also provides insight into whether the enzyme can tolerate groups of larger steric bulk at that position[144]. Carbohydrates with an acetamido group at the 2-position (GlcNAc and GalNAc) are widely occurring structures in mammalian systems[34]. We synthesized these acetamido derivatives both to investigate the effect of this group at other positions on the ring, as well as to further probe the steric constraints of the enzyme. Additionally, if the enzyme would tolerate the acetamido derivative it would probably also tolerate a bromo- or iodoacetamido derivative which could act as an alkylating agent for the postulated active site nucleophile.

Another probe of steric constraints involved the synthesis of compounds where the configuration of the 3, 4 diol had been altered, thus compounds containing the 3 and 4 epimers of galactose, i.e., gulose (**12, 30**) and glucose (**13, 31**) were prepared. Such compounds could be used to probe for van der Waals stacking interactions in the active site. In particular, we knew that the conformation of **2** was such that the fucose was positioned over the top face of the galactose residue[111]. The bottom face of the β-linked galactose, containing axially oriented hydrogens at C-1, C-3 and C-5 would provide a hydrophobic surface that could be involved in this kind of stacking interaction. Finally, spurred by the previously mentioned report[147] that a L-arabinose binding protein also recognizes D-galactose, the L-arabinose derivative (**20, 38**), was synthesized.

CHAPTER TWO:

RECOGNITION OF SYNTHETIC DISACCHARIDE ANALOGS OF THE ACCEPTOR α-L-Fuc*p*-(1→2)-β-D-Gal*p*-OR BY THE BLOOD-GROUP A AND B GENE-SPECIFIED GLYCOSYLTRANSFERASES

2. 1 INTRODUCTION

This chapter discusses the preparation and biochemical testing of disaccharide analogs of **2**. The first part of the chapter details the experimental work carried out to synthesize and biochemically evaluate these analogs. In the second part, I present an analysis of the results and propose a number of interactions that might be important for recognition. Finally, I briefly discuss some ideas for exploration in the future.

2. 2 CHEMICAL SYNTHESIS OF DISACCHARIDE ANALOGS

The chemical synthesis of disaccharides **2-20**, required as active-site probes for the A- and B-transferases, followed established synthetic strategy. As mentioned in the introduction (Fig 25), in order to easily prepare analogs for all three enzymes (A, B and H), the general synthetic scheme chosen involved first the synthesis of a suitably protected galactose residue with the OH-2 free. To prepare the disaccharide analogs, this alcohol was fucosylated under halide ion glycosylation conditions[157] and then deprotected to give the final products (Fig 27).

The retrosynthetic analysis leading to the monosaccharide precursors required for fucosylation is presented in Fig 28. The fucosyl acceptors chosen are shown as **A** (Gal 6-OH modified derivatives), **B** (Gal 4-OH modified derivatives) and **C** (Gal 3-OH modified derivatives). Each of these could, in turn, be prepared by replacement of the corresponding hydroxy analog with the appropriate functional group. Hence, alcohols **57** and **52** would serve as direct precursors. For those analogs modified at Gal 3-OH,

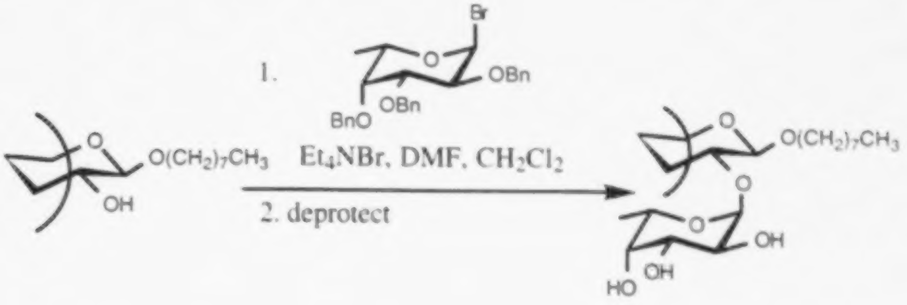

1.
Br
OBn
BnO
OBn
O(CH2)7CH3
Et4NBr, DMF, CH2Cl2
2. deprotect
OH
O(CH2)7CH3
OH
HO
OH

Fig 27. Fucosylation method used for disaccharide synthesis.

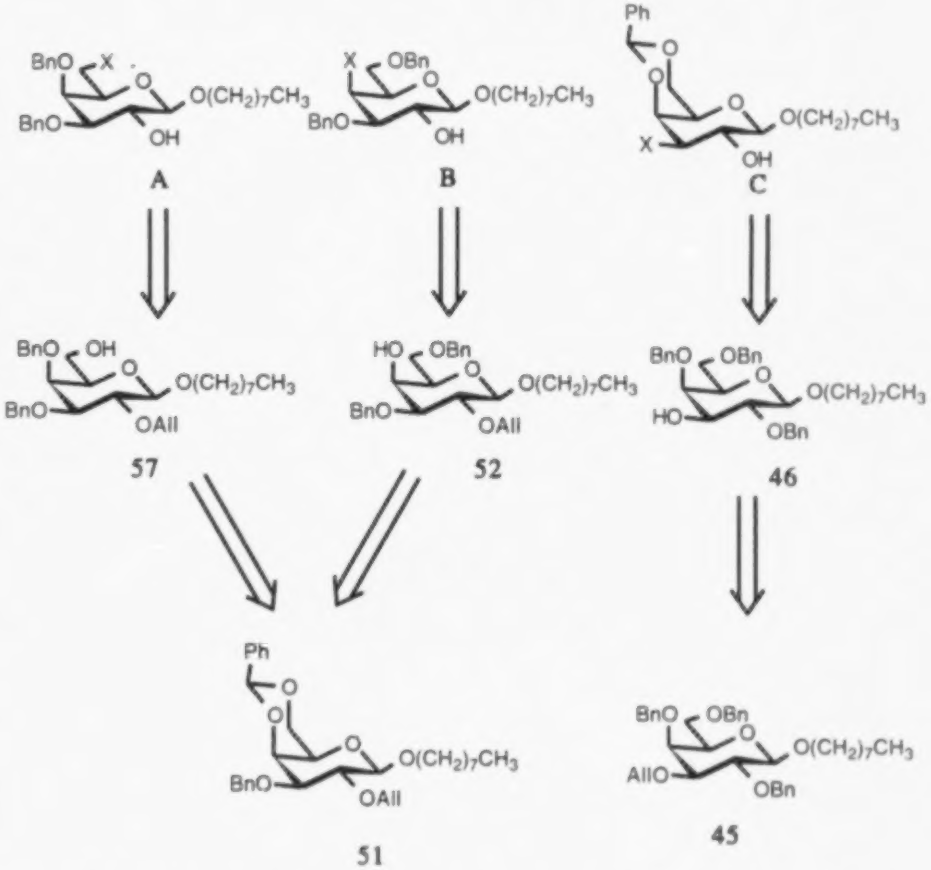

BnO
X
O(CH2)7CH3
OH
A
X
OBn
B
Ph
C
OH
57
OAll
52
HO
46
51
AllO
45

Fig 28. Retrosynthetic analysis and required monosaccharide precursors for the synthesis of **A**-**C**. X = H, OCH_3, F or protected N.

further manipulations after functional group replacement (hydrogenolysis and benzylidenation) would be needed to convert alcohol precursor **46** to fucosyl acceptor **C**. The alcohols with free 4- and 6-OH groups (**57** and **52**) could be readily prepared from benzylidene **51** via regioselective acetal ring opening. Finally, **46** could be prepared by the deallylation of the fully protected derivative **45**.

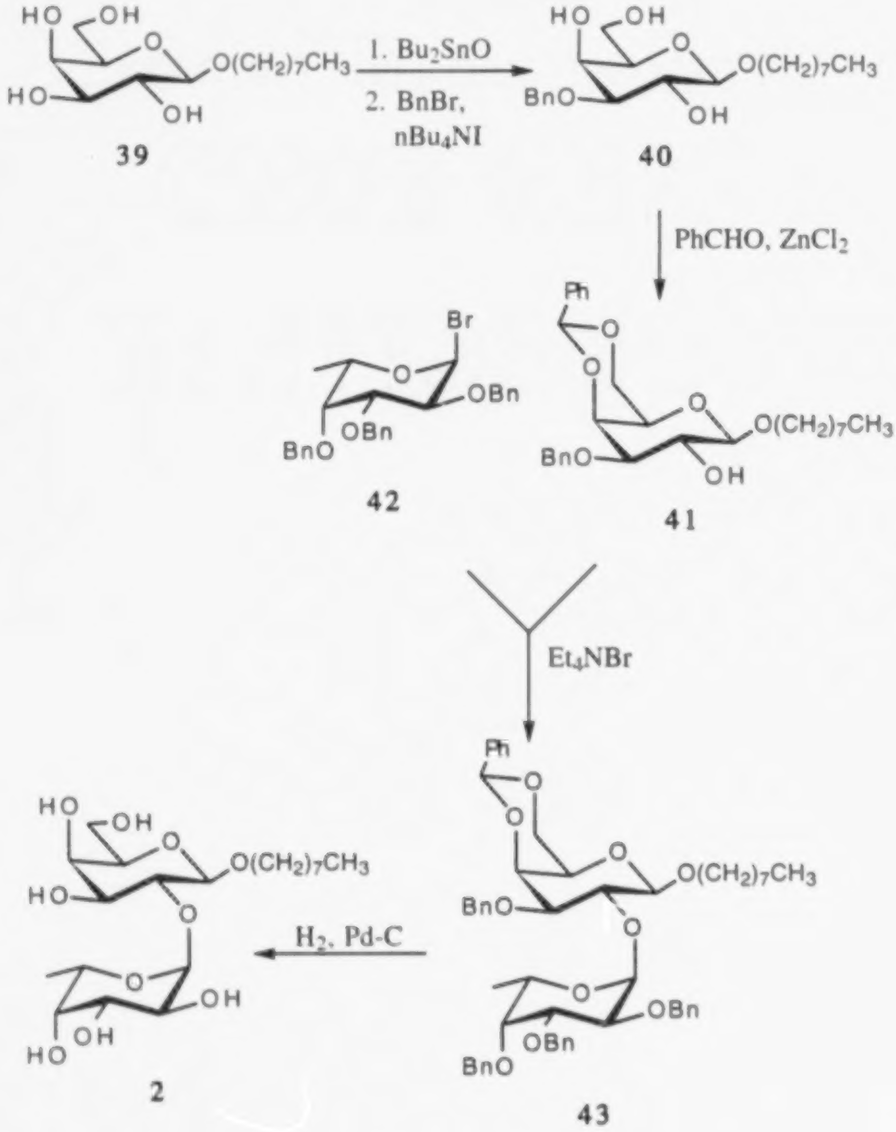

HO
OH
O(CH2)7CH3
HO
OH
39
1. Bu2SnO
2. BnBr, nBu4NI
BnO
40
PhCHO, ZnCl2
Ph
Br
OBn
BnO
42
41
Et4NBr
H2, Pd-C
2
43

Fig 29. Preparation of the native disaccharide **2**.

2. 2. 1 PREPARATION OF NATIVE DISACCHARIDE

Preparation of the unmodified acceptor **2** (Fig 29) began with the regioselective benzylation of octyl β-D-galactopyranoside[158], **39**, with dibutyltin oxide and benzyl bromide to afford **40** in a 60% yield. Benzylidenation of **40** by reaction with zinc chloride and benzaldehyde gave **41** (86%) which was fucosylated with **42**[174] to give the protected disaccharide **43** (67%). Hydrogenation of **43** gave **2** (90%).

2. 2. 2 PREPARATION OF DEOXY ANALOGS

The 3-deoxy analog was prepared (Fig 30) by regioselective allylation of **39**, under conditions similar to those for the synthesis of **40**, to give **44** (60%). The remaining hydroxyl groups were protected as benzyl ethers and then the allyl group was removed to provide **46** (70% over two steps). Formation of the xanthate **47** (86%), radical deoxygenation and hydrogenation gave octyl 3-deoxy-β-D-xylo-hexopyranoside **21** (two steps 62%). Benzylidenation of **21** with zinc chloride and benzaldehyde gave **49** (83%) which was converted to **3** by fucosylation and subsequent deprotection (76% from **49**).

The synthesis of the 4-deoxy derivative began (Fig 31) with allylation of **41** to give **51** (90%). Regioselective benzylidene ring opening with sodium cyanoborohydride afforded the 4-hydroxy derivative **52** (81%) which was then converted to the xanthate **53** (80%). Radical reduction proceeded in good yield (85%) to give **54**. Deallylation then provided **55** (86%) which was ready for fucosylation. After fucosylation it was not possible to obtain the protected disaccharide (**56**) in pure form. The partially purified product was therefore debenzylated and the purified product **4** (39% from **55**) characterized.

As opposed to the preparation of the 3 and 4-deoxy derivatives, the synthesis of the 6-deoxy analog proved more problematic (Fig 32). Initial attempts began with the benzylidene derivative **51** which was converted to the alcohol **57** by treatment with aluminum trichloride and lithium aluminum hydride. This alcohol could be converted to

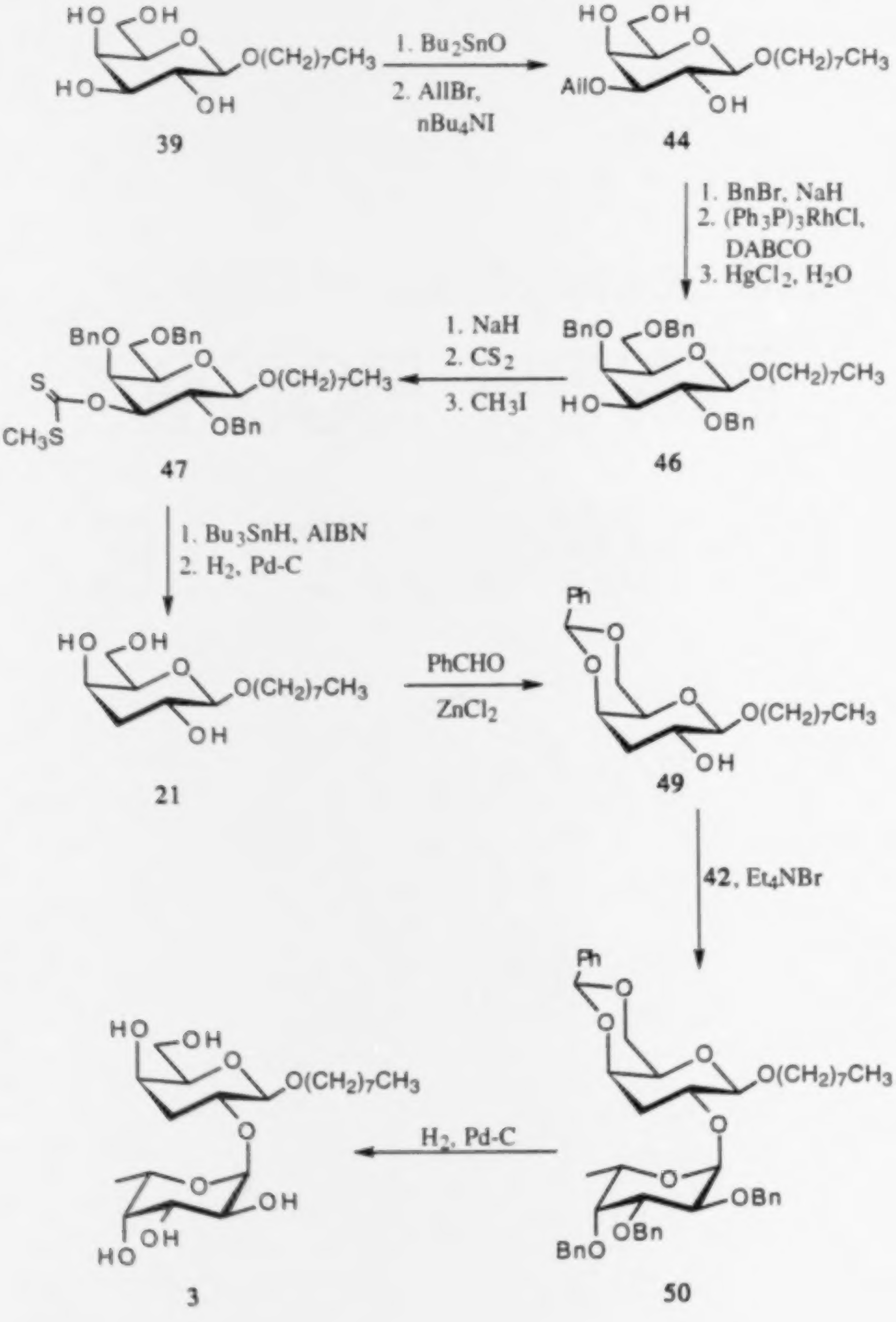

39
1. Bu$_2$SnO
2. AllBr, nBu$_4$NI
44
1. BnBr, NaH
2. (Ph$_3$P)$_3$RhCl, DABCO
3. HgCl$_2$, H$_2$O
46
1. NaH
2. CS$_2$
3. CH$_3$I
47
1. Bu$_3$SnH, AIBN
2. H$_2$, Pd-C
21
PhCHO
ZnCl$_2$
49
42, Et$_4$NBr
50
H$_2$, Pd-C
3
HO
OH
O
O(CH$_2$)$_7$CH$_3$
AllO
BnO
OBn
HO
S
CH$_3$S
Ph
OH

Fig 30. Preparation of the 3-deoxy disaccharide 3.

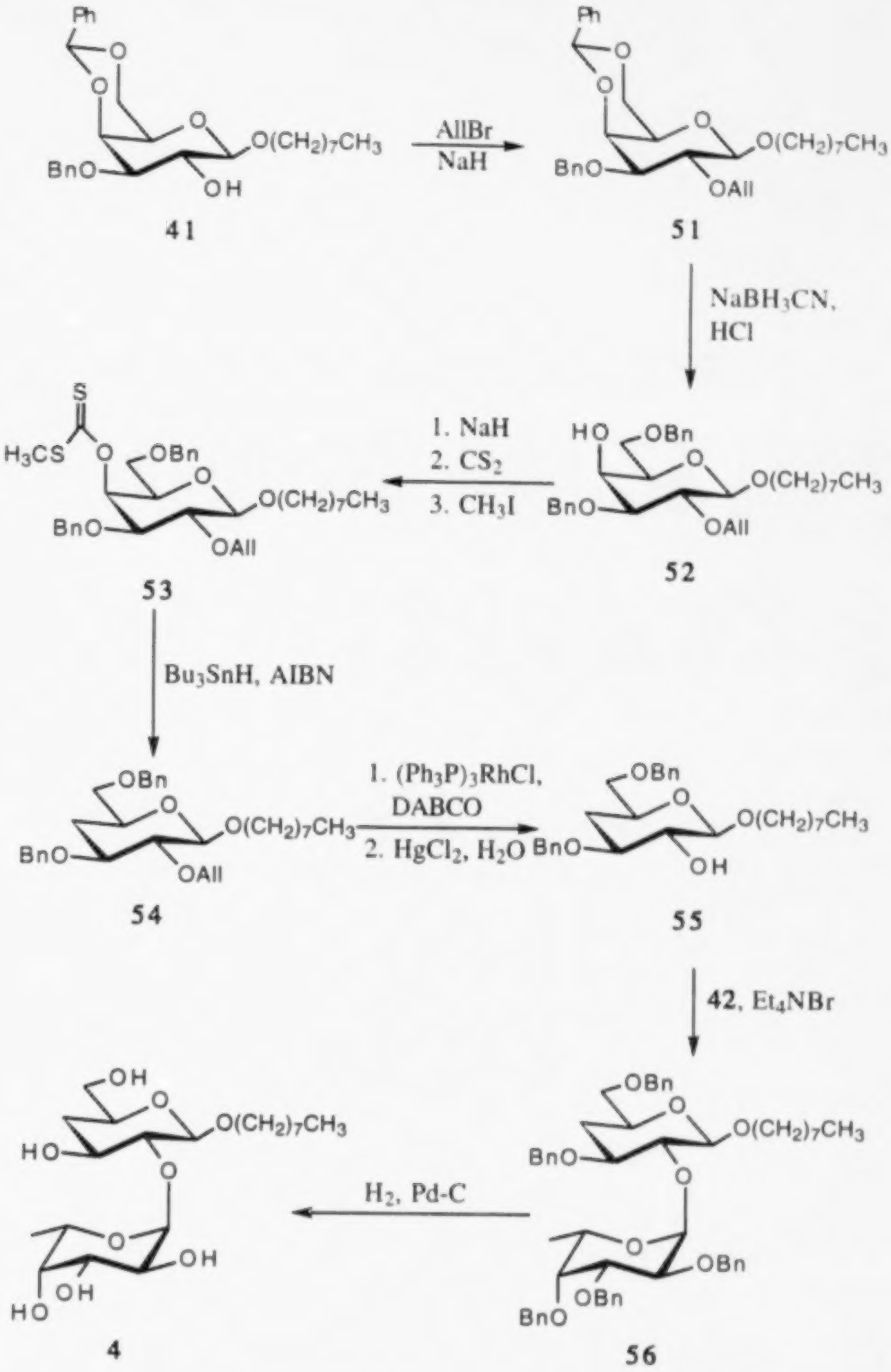
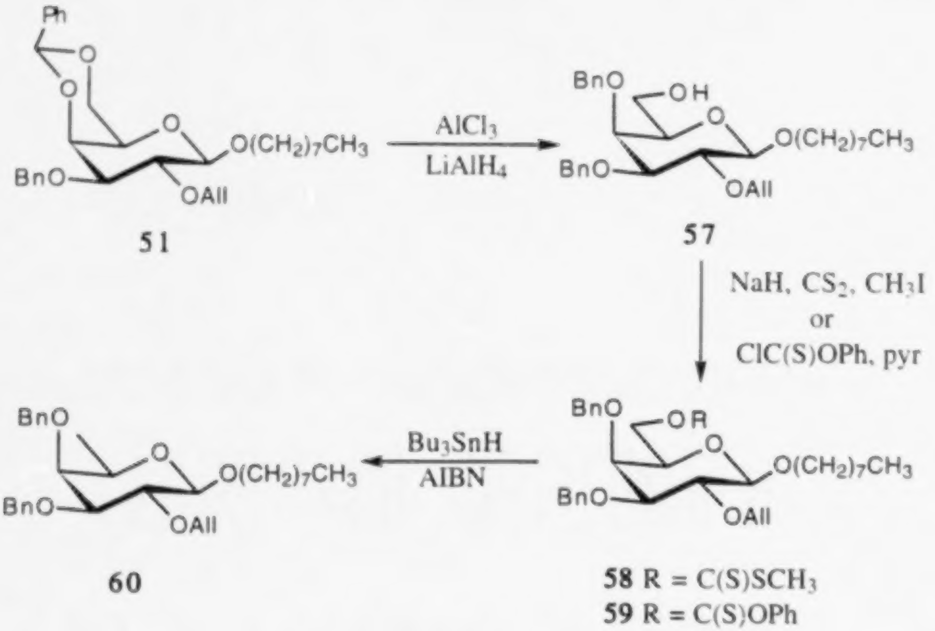
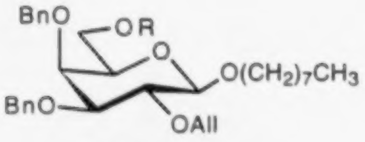

AllBr
NaH
NaBH3CN,
HCl
1. NaH
2. CS2
3. CH3I
Bu3SnH, AIBN
1. (Ph3P)3RhCl,
DABCO
2. HgCl2, H2O
42, Et4NBr
H2, Pd-C
41
51
52
53
54
55
56
4
Ph
O
OH
BnO
OAll
O(CH2)7CH3
HO
OBn
H3CS
S

Fig 31. Preparation of the 4-deoxy disaccharide **4**.


Ph
O
O
O
BnO
O(CH$_2$)$_7$CH$_3$
OAll
51
AlCl$_3$
LiAlH$_4$
BnO
OH
O
O(CH$_2$)$_7$CH$_3$
BnO
OAll
57
NaH, CS$_2$, CH$_3$I
or
ClC(S)OPh, pyr
BnO
O
O(CH$_2$)$_7$CH$_3$
BnO
OAll
60
Bu$_3$SnH
AIBN
BnO
OR
O
O(CH$_2$)$_7$CH$_3$
BnO
OAll
58 R = C(S)SCH$_3$
59 R = C(S)OPh


BnO
OR
O
O(CH$_2$)$_7$CH$_3$
BnO
OAll

61 R = Ts
62 R = Tf

Fig 32. Attempts to prepare the 6-deoxy disaccharide **5**.

either the xanthate (**58**) or the phenylthiocarbonyl derivative (**59**) in good yield, however attempts to deoxygenate these compounds using the prescribed radical methods[159] gave only very low yield (12%) of the product **60**. The low yields are due presumably to the competition between radical cleavage leading to the alcohol[160], and deoxygenation, as TLC of the reaction mixtures showed that a significant amount of the 6-hydroxy derivative had been formed. Another route to the compound, involving tosylate displacement was explored. However, formation of the 6-O-tosyl derivative **61** was very sluggish and the subsequent hydride displacement to **60** proceeded again in low yield (20%). Attempts to prepare the more reactive triflate **62** failed as well, as only decomposition was detected by TLC.

Finally, the synthesis could be completed by taking another approach starting with methyl glycoside **63** (Fig 33). Benzylidene ring opening of **63** with N-bromosuccinimide and subsequent reduction of the 6-bromo adduct **64**, gave the methyl 6-deoxy galactoside derivative **65** in 69% overall yield. Acetylation (96%) followed by treatment of **66** with dichloromethyl methyl ether, as described by Glaudemans and Kovac[161], gave chloride **67** (78%) which was glycosylated with octanol, using silver triflate activation, to provide **68** (88%). This fully protected derivative was converted to alcohol **69** (90%) by selective deacetylation with methanolic hydrogen chloride. Fucosylation and then deprotection afforded the 6-deoxy derivative **5** (65%, 2 steps).

2. 2. 3 PREPARATION OF FLUORO ANALOGS

In order to prepare the 3-fluoro analog a synthesis starting with alcohol **46** was initially chosen. It was hoped that the 3-fluoro derivative could be prepared using a double inversion route at C-3, via the 3-epimer of galactose, gulose. To this end, triflate **71** was prepared from **46** (Fig 34). The displacement of the triflate with sodium benzoate was attempted, but the yield of the desired product **72**, was very low (<10%). A search of the literature uncovered a paper that reported the difficulty of such displacements, e.g., conversion of galactopyranose derivatives to gulopyranose derivatives[162]. Furthermore, the paper also reported that the converse displacement (conversion of the *gulo*-configuration to the *galacto*-configuration) is also difficult, therefore this approach was abandoned.

The next attempt involved (Fig 34) the synthesis of the 3-keto derivative **73**, which was synthesized in modest yield (40%) by oxidation of **46**, with pyridinium chlorochromate. It was hoped that reduction of this ketone with sodium triacetoxy-

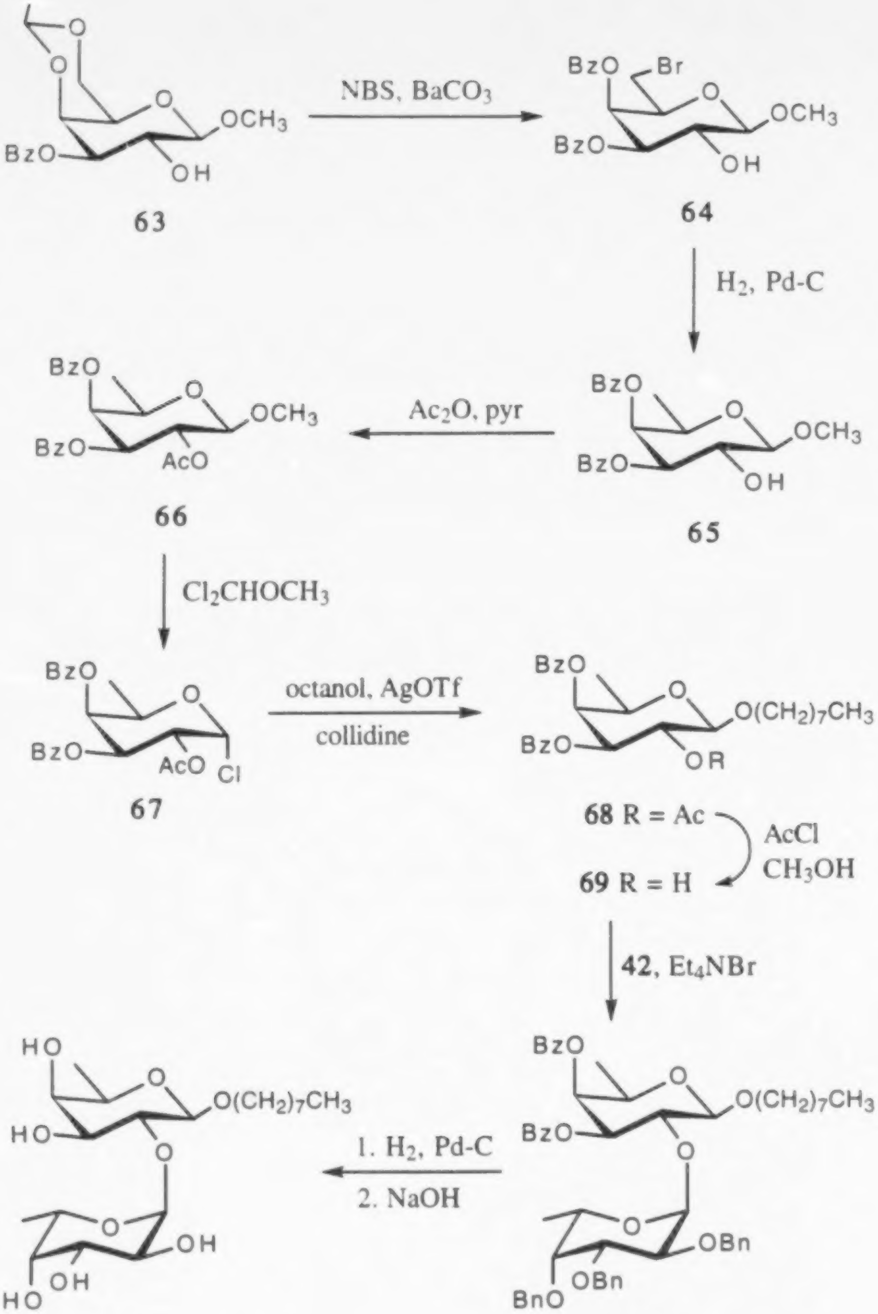
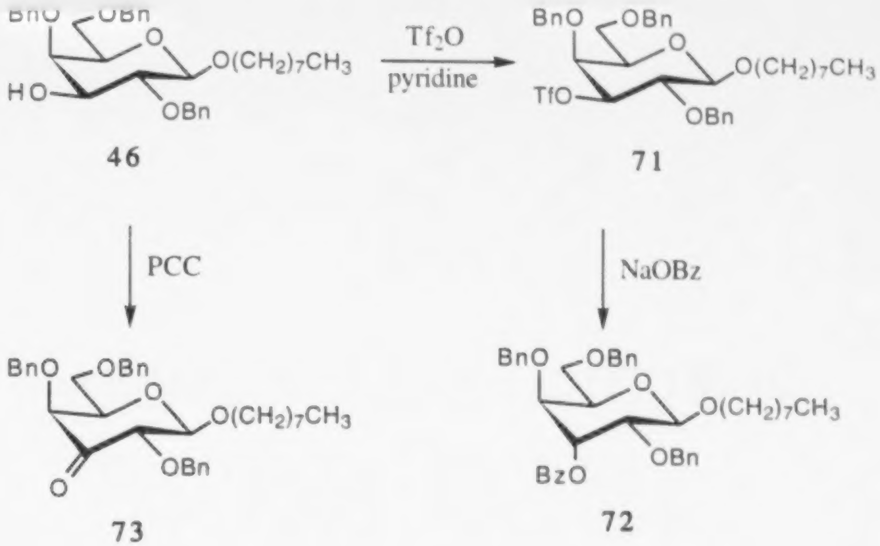
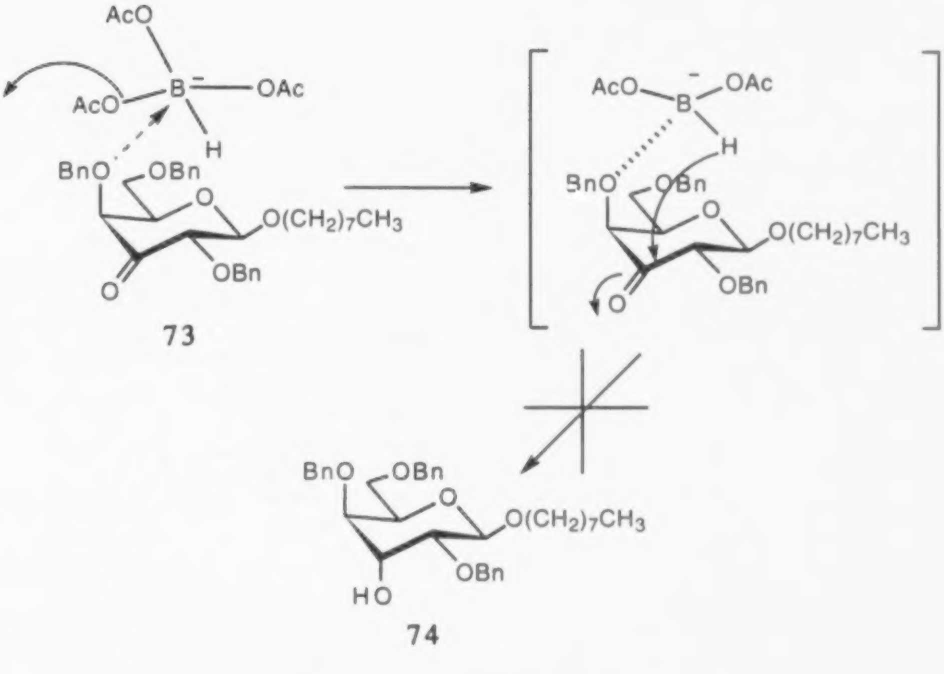

NBS, BaCO$_3$
H_2, Pd-C
Ac_2O, pyr
Cl_2CHOCH_3
octanol, AgOTf
collidine
AcCl
CH_3OH
42, Et_4NBr
1. H_2, Pd-C
2. NaOH
BzO
OCH$_3$
OH
Br
AcO
Cl
O(CH$_2$)$_7$CH$_3$
OR
HO
OBn
BnO
63
64
65
66
67
68 R = Ac
69 R = H
70
5

Fig 33. Preparation of the 6-deoxy disaccharide **5**.



BnO
OBn
O
O(CH2)7CH3
HO
OBn
46
Tf2O
pyridine
TfO
71
PCC
NaOBz
73
BzO
72

Fig 34. Attempts to prepare the 3-fluoro disaccharide **6**.



AcO
AcO
B
OAc
H
BnO
OBn
O
O(CH2)7CH3
OBn
O
73
AcO
B
OAc
H
BnO
OBn
O
O(CH2)7CH3
OBn
O
BnO
OBn
O
O(CH2)7CH3
OBn
HO
74

Fig 35. Expected pathway for the reduction of ketone **73** with sodium triacetoxy borohydride.

borohydride would give the desired *gulo*-configuration derivative. This reducing agent had been previously reported[163] to give high stereoselectivity of the trans diol via complexation with a neighboring hydroxyl group and delivery of the hydride from the same side of the molecule. In the present case, it was hoped that the reducing agent would complex with the benzyl ether oxygen axially positioned at C-4. Delivery of the hydride at C-3 from the same side of the molecule would lead to the diaxial alcohol, **74** (Fig 35). However, upon treatment of the ketone with either this reagent or the more commonly used sodium borohydride a quantitative yield of the *galacto*-isomer was obtained. Obviously the stereochemistry of this reduction is dictated by steric hindrance. Since the top side of the molecule is more sterically congested due to both the axial benzyl ether at C-4 and the β-glycoside, the hydride is delivered from the bottom face leading to the *galacto*-derivative.

At this point we chose a longer synthesis, a portion of which had been previously reported[164] (Fig 36). Reaction of the acetylated derivative[164] **75** with hydrogen bromide in acetic acid gave bromide **76** (80%) which was reacted with octanol and silver triflate to give glycoside **77** (62%). Zemplen deacetylation produced **24** which was then converted to benzylidene acetal **78** (90% over two steps). The synthesis of the 3-fluoro derivative **6** was completed by fucosylation of **78** (89%) followed by hydrogenation (82%).

As in the case of the 3-fluoro derivative, it was hoped that a double inversion strategy would yield the 4-fluoro derivative (Fig 37 A). Inversion of alcohol **52** to the benzoate **80**, proceeded in good yield via displacement of the 4-triflate to give the *gluco* configuration benzoate in 74% yield. Deprotection with sodium methoxide afforded glucoside **81**. Treatment of **81** with a commonly used fluorinating reagent, diethyl amino sulfur trifluoride (DAST), resulted not only in very low yields (< 5%) of the fluorinated product **82**, but also the formation of a two major, non-fluorinated side products. Although no attempt was made to determine the structures of the side products

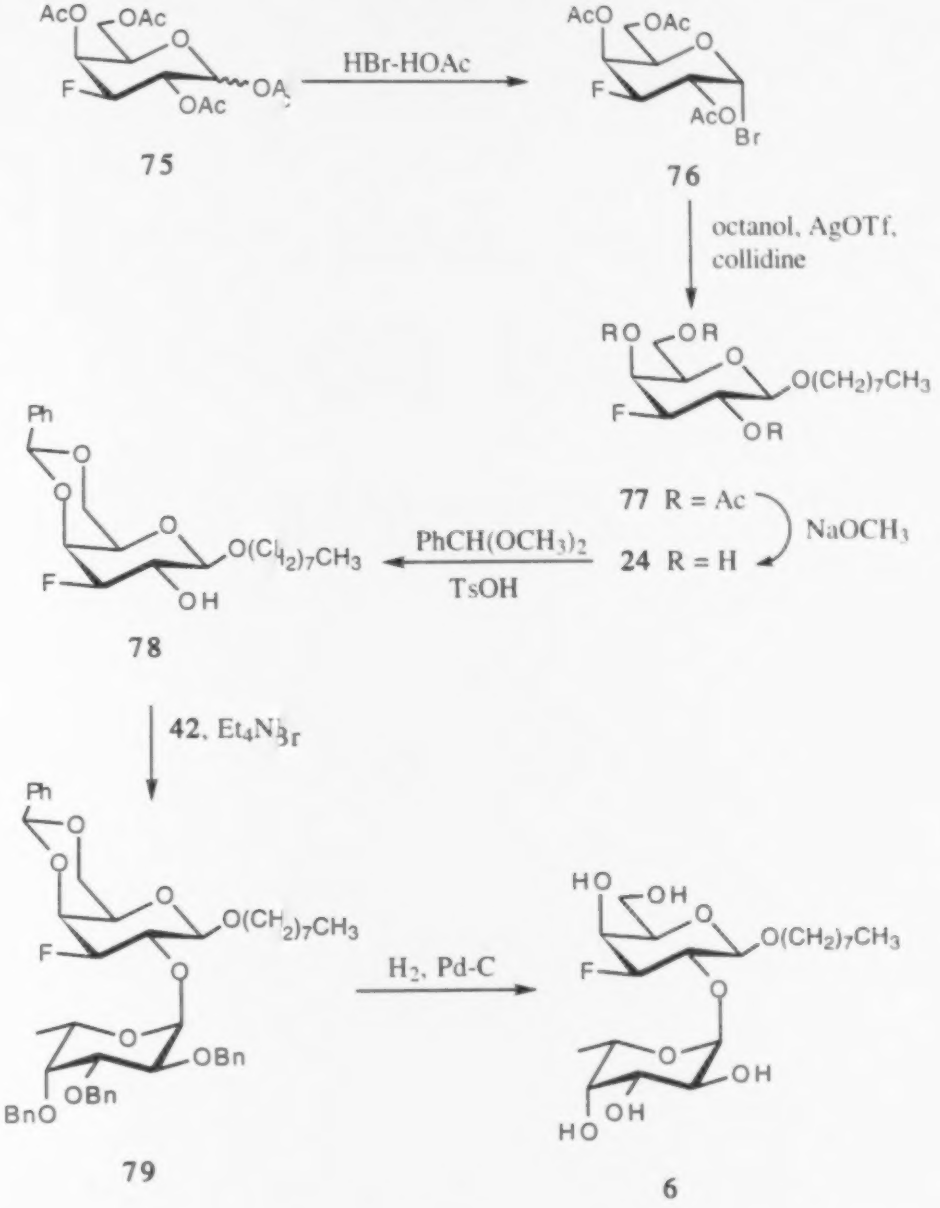

AcO
OAc
O
F
OAc
OAc
75
HBr-HOAc
AcO
OAc
O
F
AcO
Br
76
octanol, AgOTf,
collidine
RO
OR
O
O(CH2)7CH3
F
OR
77 R = Ac
24 R = H
NaOCH3
PhCH(OCH3)2
TsOH
Ph
O
O
O
O(CH2)7CH3
F
OH
78
42, Et4NBr
Ph
O
O
O
O(CH2)7CH3
F
O
O
OBn
OBn
BnO
79
H2, Pd-C
HO
OH
O
O(CH2)7CH3
F
O
O
OH
OH
HO
6

Fig 36. Preparation of the 3-fluoro disaccharide **6**.

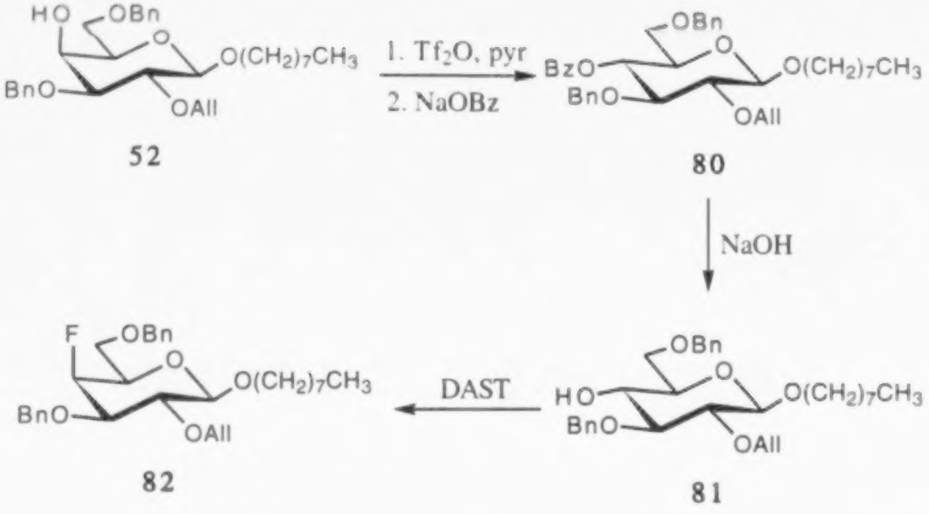

HO
OBn
O
O(CH2)7CH3
BnO
OAll
52
1. Tf2O, pyr
2. NaOBz
BzO
80
NaOH
F
82
DAST
HO
81

Fig 37 A. Attempts at preparing the 4-fluoro disaccharide.

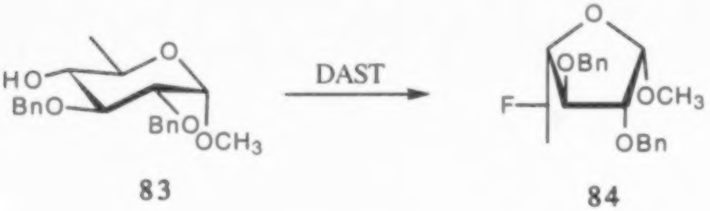

HO
BnO
O
BnO
OCH3
83
DAST
O
OBn
F
OCH3
OBn
84

Fig 37 B. Formation of furanose side product during DAST treatment.

formed, a recent report on a similar molecule reported that fluorination was inefficient and that one of the side products produced in that reaction was a ring contraction adduct (**83**→**84**)[165] The authors reported only a 2% yield of fluorinated product compared with a 38% yield of the contraction product. An analogous product could have been produced in this reaction. The formation of elimination products during reactions with DAST has also been reported with similar molecules[166]. Fluorination via the triflate of alcohol **81** by reaction with tetrabutylammonium fluoride gave better, but still modest yields of the product **82** (48%), and moreover the product was difficult to purify. Despite repeated attempts to purify the compound, a pure product could not be obtained.

Furthermore, the amount of product lost during the purification attempts was sizable and hence there was insufficient compound with which to continue. Thus another route to **7** was chosen as described below.

The synthesis of the 4-fluoro derivative (Fig 38) began with the commercially available octyl β-D-glucopyranoside (**31**) which was converted to the fully protected derivative **85** in two steps (76%). Benzylidene ring opening as described for the conversion of **51** to **52**, gave the 4-hydroxy derivative **86** (88%). Alcohol **86** was fluorinated, via its 4 triflate, by reaction with tetrabutylammonium fluoride (80%). Fluoro-galactoside **87** was converted to **25** by hydrogenation (85%) and then protected as the dibenzoate **89** using dibutyltin oxide and benzoyl chloride (37%). A significant amount (50%) of the monobenzoate **88** was also present, but this could be easily converted to **89** by treatment with benzoyl chloride and pyridine (76%). To complete the synthesis, alcohol **89** was fucosylated and deprotected to afford **7** (74%, over two steps).

Initial attempts to prepare the 6-fluoro derivative involved the replacement of the 6-OH of **57** by treatment with DAST (Fig 39). However, when this reaction was carried out, no fluorinated product **91** was detected. The major product (60%) appeared by ^{1}H NMR to be an anhydro derivative of the molecule **57**. A previous publication[167] reports the formation of the 3,6-anydro derivative **93** during attempts to fluorinate the 6 position of galactose derivative **92** with DAST. The exact structure of the by-product formed from **57** was not determined; however, an anhydro derivative similar to **93** could have been produced. Formation of a 4,6 anhydro product is also possible. Faced with this intramolecular reaction problem, due to the ability of the molecule to change conformation, we chose a strategy starting with a compound unable to change conformation as shown in Fig 40.

Bromide **96** was prepared by treatment of 6-deoxy-6-fluoro-1,2:3,4 di-O-isopropylidene-α-D-galactopyranose[168] (**94**) first with 90% trifluoroacetic acid, then

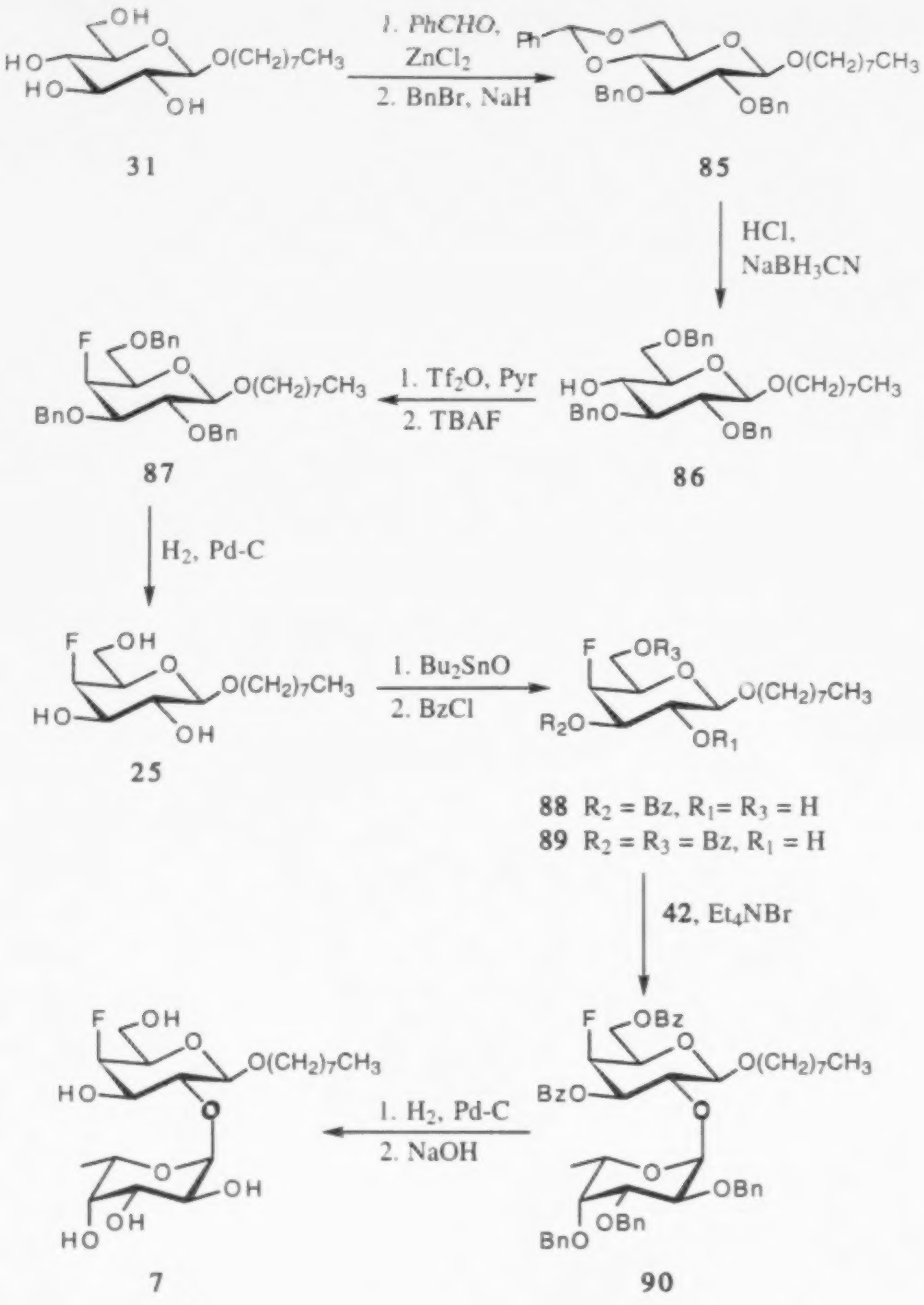
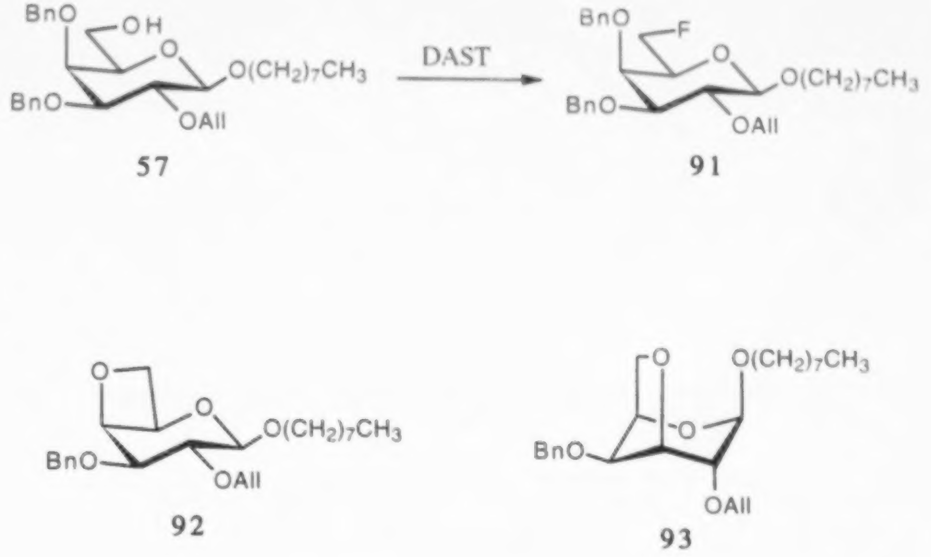

1. PhCHO,
ZnCl2
2. BnBr, NaH
31
85
HCl,
NaBH3CN
1. Tf2O, Pyr
2. TBAF
87
86
H2, Pd-C
1. Bu2SnO
2. BzCl
25
88 R2 = Bz, R1= R3 = H
89 R2 = R3 = Bz, R1 = H
42, Et4NBr
1. H2, Pd-C
2. NaOH
7
90
O(CH2)7CH3
OBn
BnO
OBz
BzO
F
OH
HO
OR1
OR3
R2O
Ph

Fig 38. Preparation of the 4-fluoro disaccharide **7**.



57 91 92 93

Fig 39. Attempts at forming the 6-fluoro disaccharide.

acetic anhydride and pyridine and finally hydrogen bromide in acetic acid (three steps, 67%). Glycosylation (73%) and then Zemplen deacetylation (90%) gave **26**. The 3,4 diol was protected as a benzylidene acetal by treatment with dimethoxytoluene and toluenesulfonic acid to give **98** as a mixture of easily separable diastereomers (85%). Fucosylation of one of the diasteriomers of **98** (90%) and deprotection gave the 6-fluoro analog **8** (90%).

2. 2. 4 PREPARATION OF O-METHYL ANALOGS

The synthesis of the 3-O-methyl derivative began with alcohol **46** which was O-methylated and hydrogenated to provide octyl 3-O-methyl-β-D-galactopyranoside (**27**) in 80% yield over two steps (Fig 41). Protection of the 4,6-diol by benzylidenation gave alcohol **101** (72%). Fucosylation of **101** with **42** gave **102** which was deprotected by hydrogenation providing **9** (two steps, 47%).

To prepare the 4-O-methyl derivative **10**, alcohol **52** was methylated to give the fully protected 4-O-methyl derivative **103** in 92% yield (Fig 42). Removal of the allyl

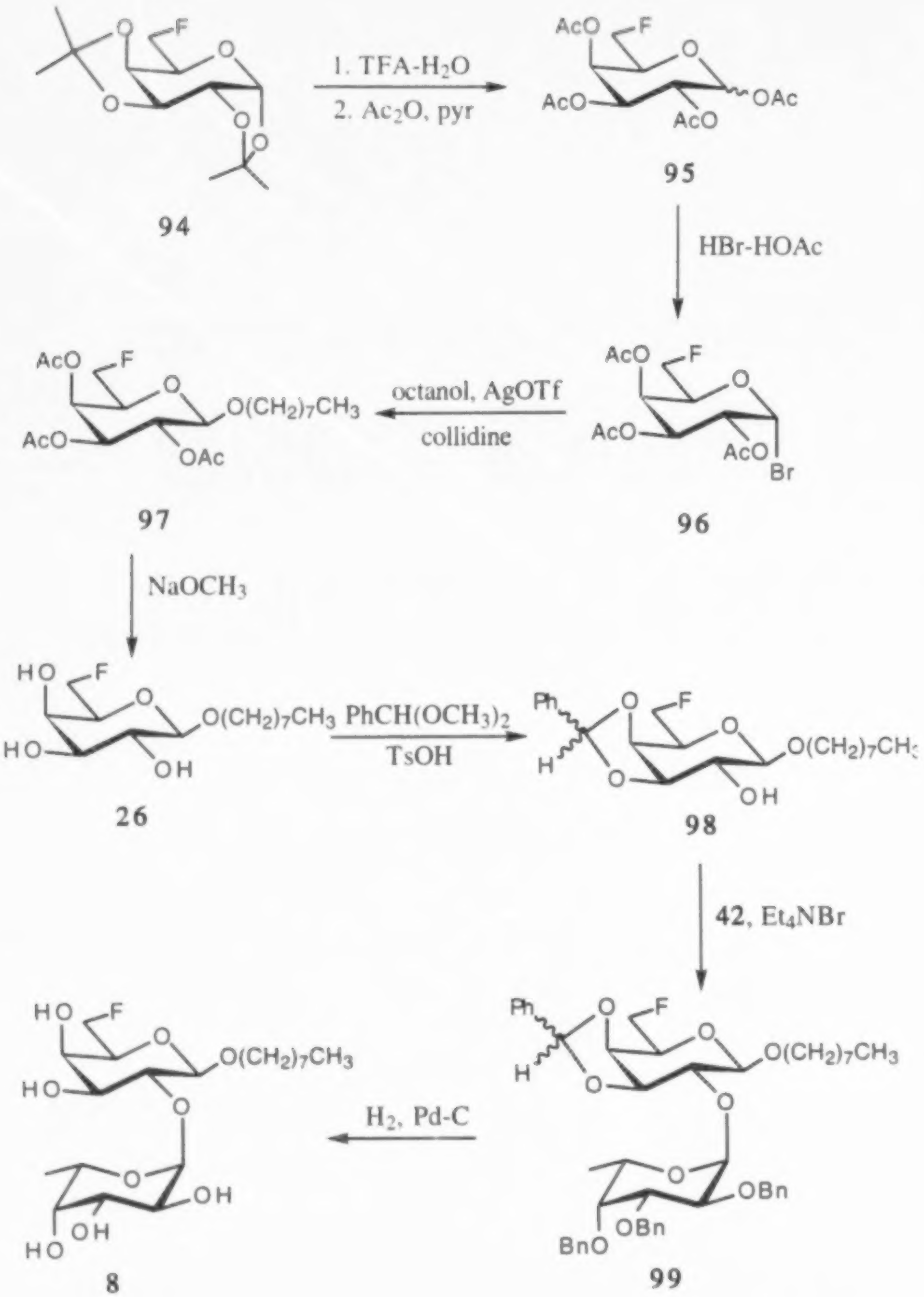

94
1. TFA-H2O
2. Ac2O, pyr
95
HBr-HOAc
96
octanol, AgOTf
collidine
97
NaOCH3
26
PhCH(OCH3)2
TsOH
98
42, Et4NBr
99
H2, Pd-C
8
AcO
OAc
F
Br
O(CH2)7CH3
HO
OH
Ph
H
OBn
BnO

Fig 40. Preparation of the 6-fluoro disaccharide **8**.

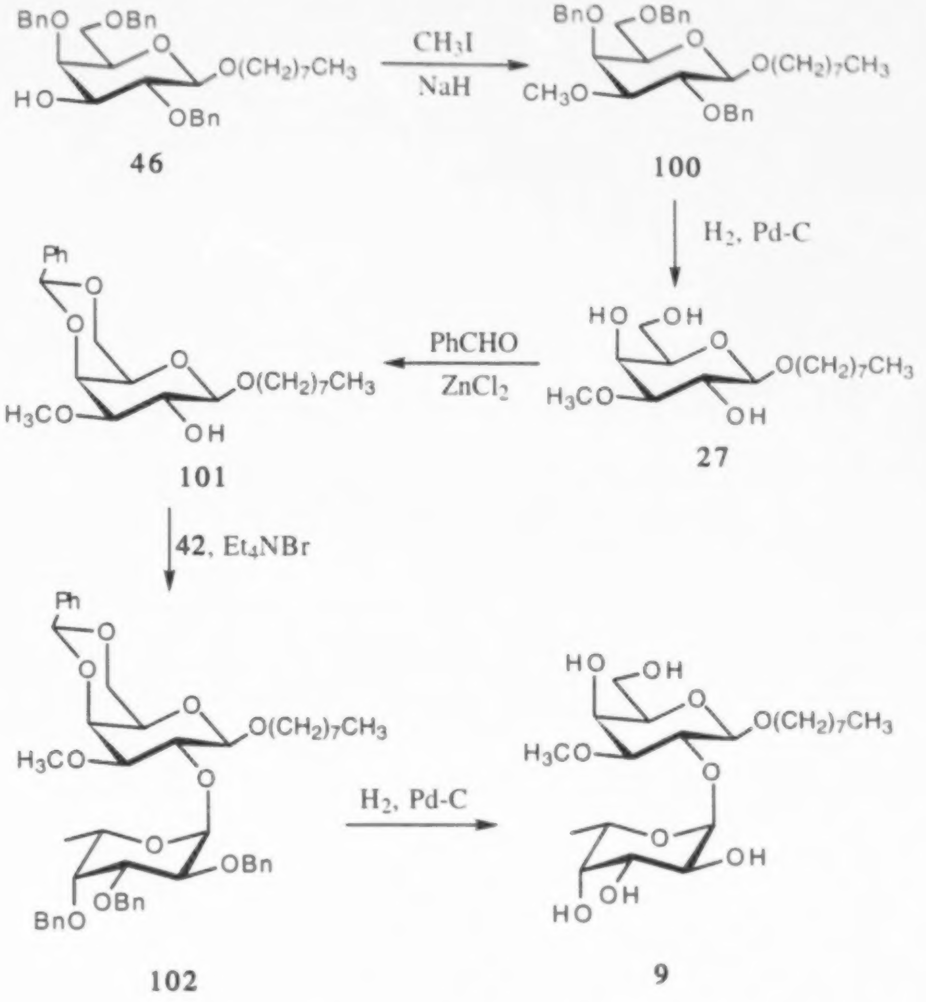

46
CH3I
NaH
100
H2, Pd-C
27
PhCHO
ZnCl2
101
42, Et4NBr
102
H2, Pd-C
9

Fig 41. Preparation of the 3-O-methyl disaccharide **9**.

group (86%) gave **104** which was fucosylated with **42**. Repeated attempts to purify the product (**105**) by column chromatography failed and the partially purified **105** was therefore directly hydrogenated and the deblocked 4-O-methyl disaccharide **10**, could thus be obtained in pure form after chromatography (30% from **104**).

The synthesis of the 6-O-methyl derivative (Fig 43) was straightforward and began with methylation of **57** by reaction with methyl iodide yielding **106** (95%).

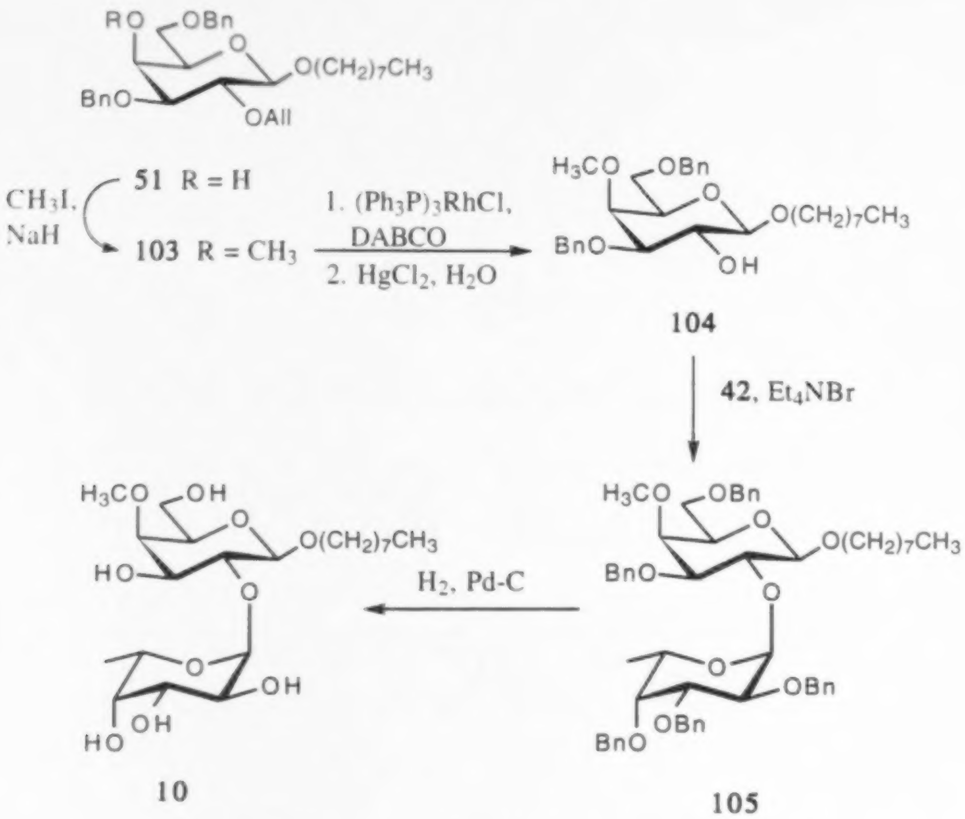

RO
OBn
O
O(CH2)7CH3
BnO
OAll
CH3I,
NaH
51 R = H
103 R = CH3
1. (Ph3P)3RhCl,
DABCO
2. HgCl2, H2O
H3CO
OBn
O(CH2)7CH3
BnO
OH
104
42, Et4NBr
H3CO
OH
O(CH2)7CH3
HO
OH
OH
HO
10
H2, Pd-C
H3CO
OBn
O(CH2)7CH3
BnO
OBn
OBn
BnO
105

Fig 42. Preparation of the 4-O-methyl disaccharide **10.**

Removal of the allyl group (81%) and fucosylation provided the protected disaccharide **108** (81%). Hydrogenation of **108** gave the desired product **11** in 93% yield (Fig 43).

2. 2. 5 PREPARATION OF EPIMERIC ANALOGS

Preparation of a 3-epimeric compound via inversion of a suitable 3-OH octyl galactoside derivative was not possible for the reasons discussed above in conjunction with the synthesis of the 3-fluoro derivative. Therefore gulose was synthesized according to a previously published method[169] and converted to the known bromide[170] **109** (Fig 44). The synthesis began with the conversion of the bromide **109** to octyl-β-D-gulopyranoside (**30**) via glycosylation and deacetylation (59% from **109**). Conversion of **30** to the diisopropylidene derivative **111** was achieved, in high yield,

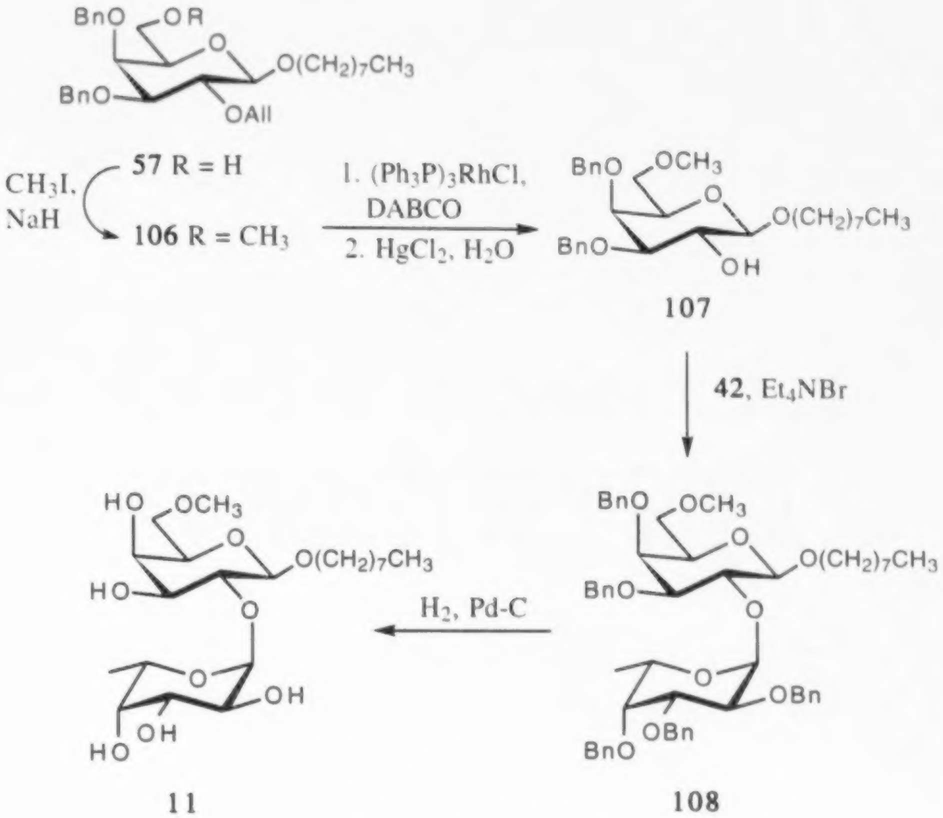

BnO
OR
O
O(CH2)7CH3
BnO
OAll
CH3I, NaH
57 R = H
106 R = CH3
1. (Ph3P)3RhCl, DABCO
2. HgCl2, H2O
BnO
OCH3
O
O(CH2)7CH3
BnO
OH
107
42, Et4NBr
HO
OCH3
O
O(CH2)7CH3
HO
O
O
OH
OH
HO
11
H2, Pd-C
BnO
OCH3
O
O(CH2)7CH3
BnO
O
O
OBn
OBn
BnO
108

Fig 43. Preparation of the 6-O-methyl disaccharide **11**.

by reaction with an excess of 2,2-dimethoxypropane (92%). Selective hydrolysis of **111** provided diol **112** which was then converted to the dibenzylated compound **113** (two steps, 59%). The isopropylidene group was removed (87%) and the resulting 2,3-diol, **114**, was transiently protected as the methyl orthoacetate. Conversion of the orthoester to the 3 acetate by reaction with acetic acid and water gave **115** (97% from **114**). Fucosylation, yielded product **116**, the purification of which was not successful. The partially purified material was therefore treated with sodium methoxide and the deacetylated product, **117**, purified and characterized (78% from **115**). Hydrogenation afforded the gulopyranose containing disaccharide **12** (91%).

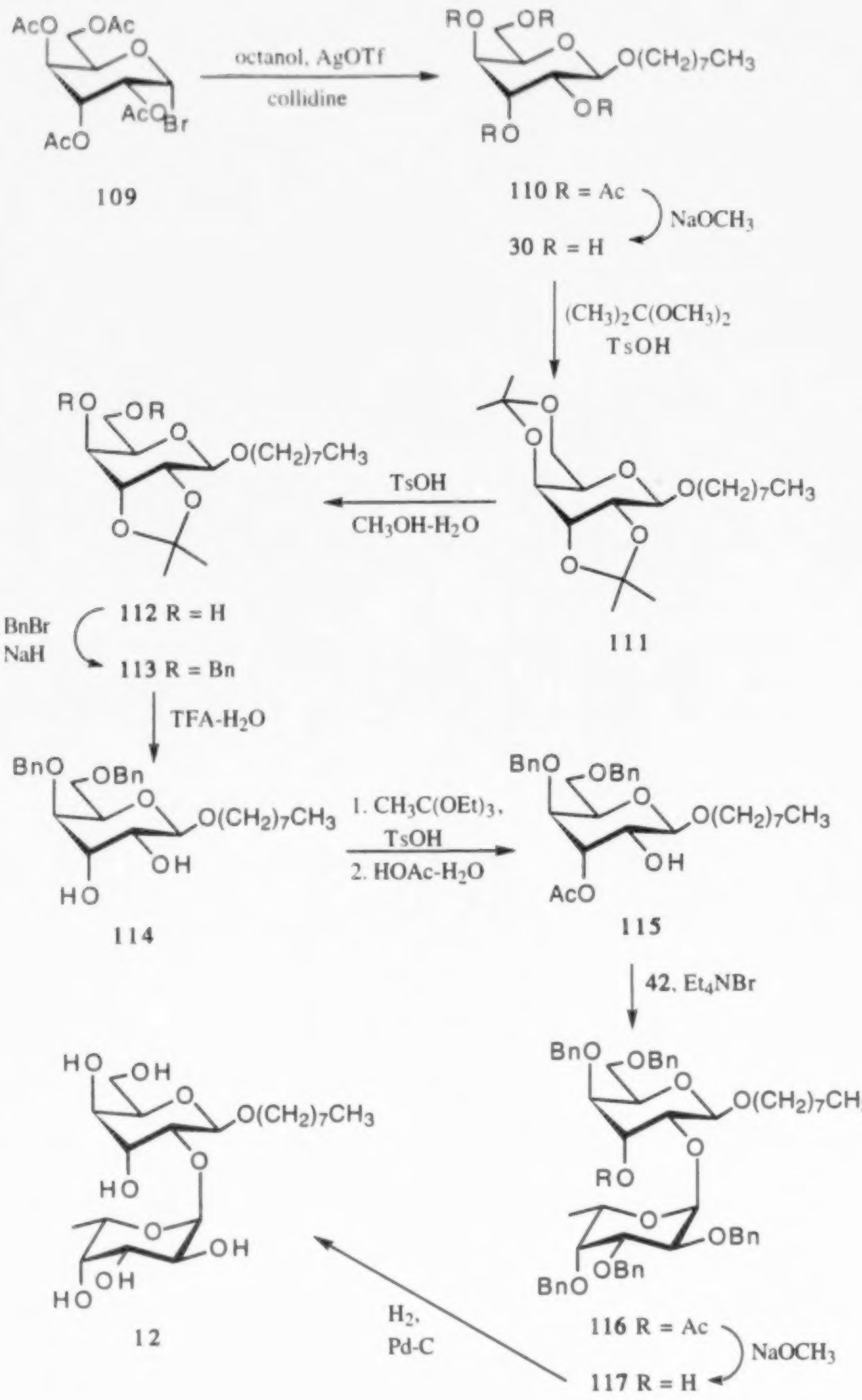

109
octanol, AgOTf
collidine
110 R = Ac
NaOCH3
30 R = H
(CH3)2C(OCH3)2
TsOH
111
TsOH
CH3OH-H2O
112 R = H
BnBr
NaH
113 R = Bn
TFA-H2O
114
1. CH3C(OEt)3, TsOH
2. HOAc-H2O
115
42, Et4NBr
116 R = Ac
NaOCH3
117 R = H
H2, Pd-C
12

Fig 44. Preparation of the 3-epimeric disaccharide **12**.

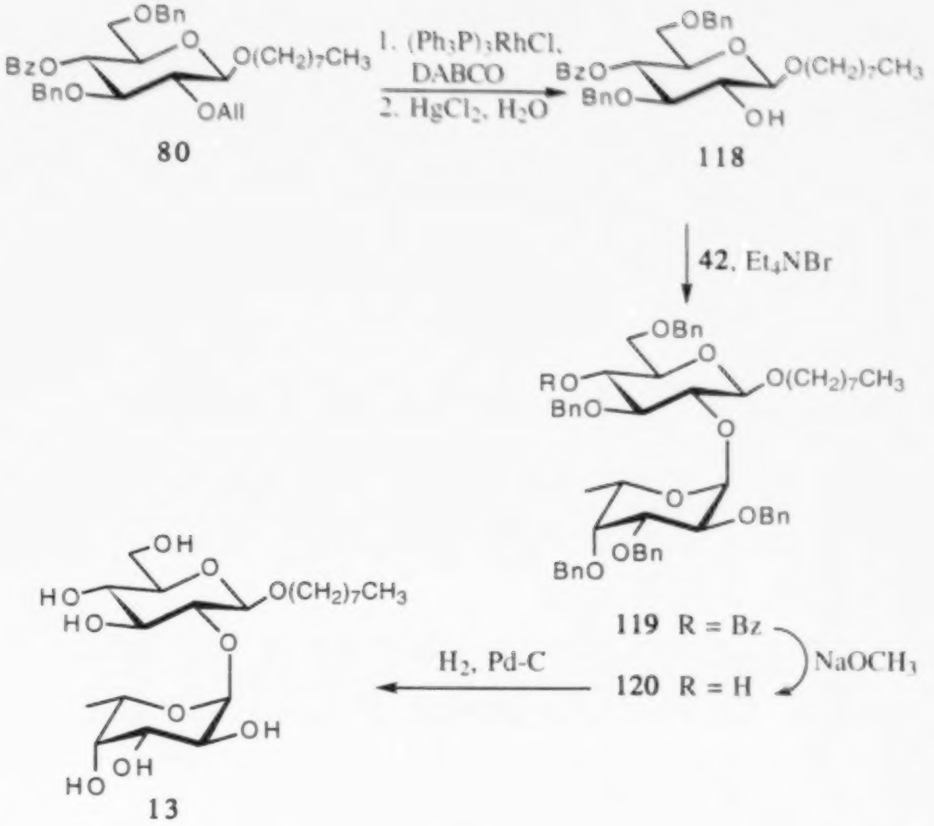

80
1. (Ph3P)3RhCl, DABCO
2. HgCl2, H2O
118
42, Et4NBr
119 R = Bz
120 R = H
NaOCH3
H2, Pd-C
13
OBn
BzO
BnO
O(CH2)7CH3
OAll
OH
RO
HO

Fig 45. Preparation of the 4-epimeric disaccharide **13**.

To obtain the disaccharide with a glucosyl moiety, (Fig 45) benzoate **80** was deallylated to give **118** (72%) and this alcohol fucosylated to give **119** which could not be purified. As described above for the synthesis of **12**, partially purified **119** was treated with sodium methoxide and then the deacylated product **120** characterized. The 4-epimeric disaccharide **13** was obtained in 80% yield from **118** after hydrogenation.

2. 2. 6 PREPARATION OF AMINO ANALOGS

Preparation of the 3-amino disaccharide by a double displacement strategy from alcohol **46**, via the gulo-configuration benzoate **72** was, as discussed above, not feasible. Therefore a synthesis beginning with the gulofuranose derivative **121**[169] was chosen (Fig 46). Reaction of the 3 triflate of **121** with sodium azide afforded azide **122**

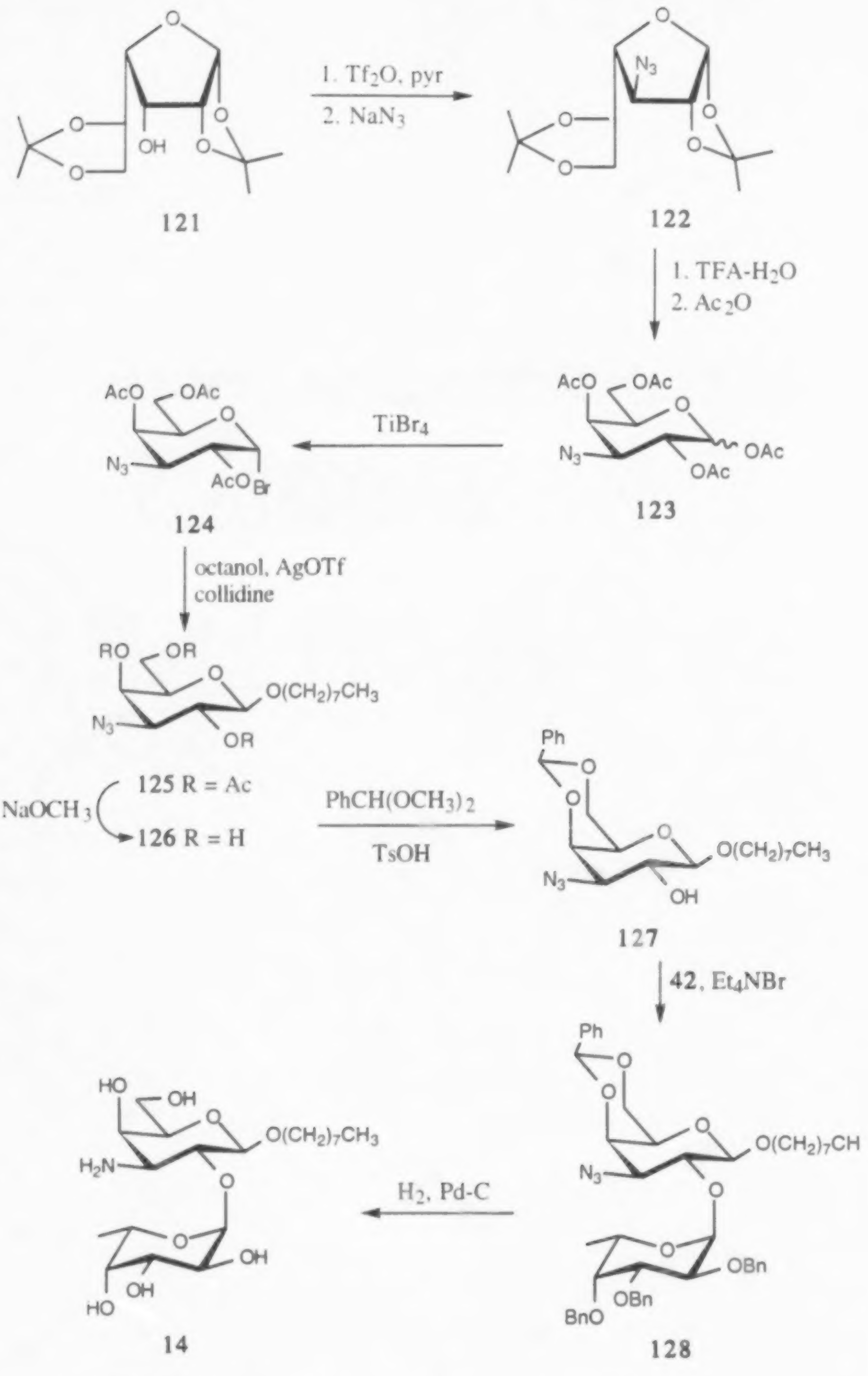
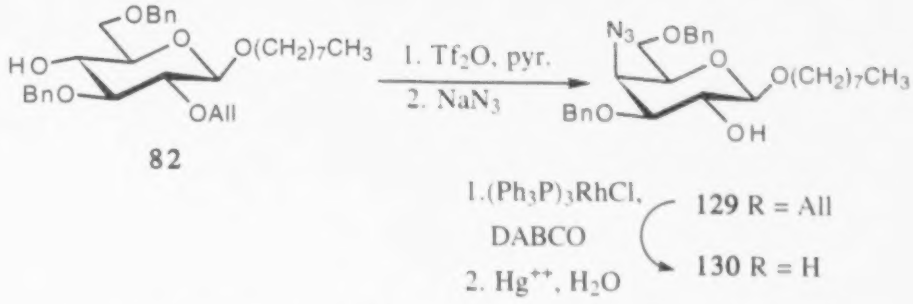

OH
121
1. Tf$_2$O, pyr
2. NaN$_3$
N$_3$
122
1. TFA-H$_2$O
2. Ac$_2$O
AcO
OAc
N$_3$
OAc
OAc
123
TiBr$_4$
AcO
OAc
N$_3$
AcO
Br
124
octanol, AgOTf
collidine
RO
OR
O(CH$_2$)$_7$CH$_3$
N$_3$
OR
125 R = Ac
NaOCH$_3$
126 R = H
PhCH(OCH$_3$)$_2$
TsOH
Ph
O(CH$_2$)$_7$CH$_3$
N$_3$
OH
127
42, Et$_4$NBr
Ph
O(CH$_2$)$_7$CH
N$_3$
OBn
OBn
BnO
128
H$_2$, Pd-C
HO
OH
O(CH$_2$)$_7$CH$_3$
H$_2$N
OH
OH
HO
14

Fig 46. Preparation of the 3-amino disaccharide **14**.



82

129 R = All

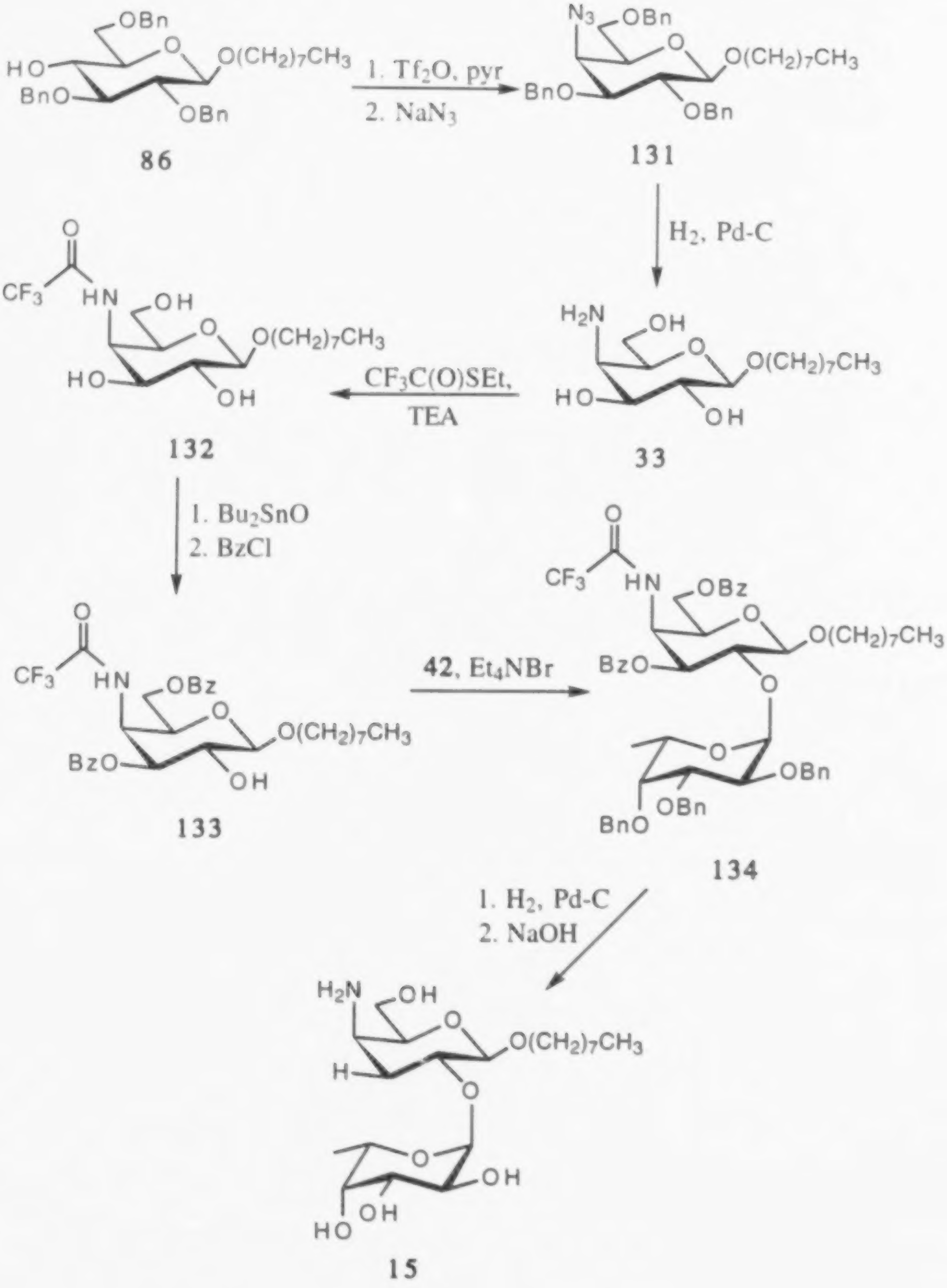
130 R = H

Fig 47. Attempts toward the preparation of the 4-amino disaccharide.

(87%). The diisopropylidene derivative was converted to the fully acetylated derivative **123** by treatment with trifluoroacetic acid followed by acetic anhydride and pyridine (56%). Bromide **124**, prepared by reaction of **123** with titanium tetrabromide, was treated with octanol to give octyl 2,4,6-tri-O-acetyl-3-azido-3-deoxy-β-D-galactopyranoside (**125**) in 63% yield. Glycoside **125** was then deacetylated and the resulting triol protected as a benzylidene acetal to provide **127** (77% from **125**). Fucosylation proceeded in modest yield (55%) to give **128** which was hydrogenated providing **14** (60%).

The preparation of the 4-amino derivative, it was envisioned, could begin with the displacement by sodium azide of the triflate of alcohol **82** (Fig 47). When attempted, this displacement proceeded in only modest yield (50%), and the product proved difficult to purify. Furthermore, the subsequent deallylation step (**129** → **130**) was accompanied by extensive decomposition. Therefore, a synthesis where the allyl group was not used was chosen (Fig 48).

Instead, the initial step in the synthesis of the 4-amino-analog was the displacement of the 4 triflate of **86** with sodium azide to give **131** (74%). Reduction of the azide and removal of the benzyl groups was achieved by hydrogenation to provide octyl 4-amino-4-deoxy-β-D-galactopyranoside (**33**, 59%). Preparation of alcohol **133** involved first protection of the amino group as a trifluoroacetate (78%) to give **132**,



OBn
HO
BnO
O
O(CH2)7CH3
OBn
86
1. Tf2O, pyr
2. NaN3
N3
OBn
O
O(CH2)7CH3
BnO
OBn
131
H2, Pd-C
O
CF3
HN
OH
O
O(CH2)7CH3
HO
OH
132
CF3C(O)SEt,
TEA
H2N
OH
O
O(CH2)7CH3
HO
OH
33
1. Bu2SnO
2. BzCl
O
CF3
HN
OBz
O
O(CH2)7CH3
BzO
OH
133
42, Et4NBr
O
CF3
HN
OBz
O
O(CH2)7CH3
BzO
O
O
OBn
OBn
BnO
134
1. H2, Pd-C
2. NaOH
H2N
OH
O
O(CH2)7CH3
H
O
O
OH
OH
HO
15

Fig 48. Preparation of the 4-amino disaccharide **15**.

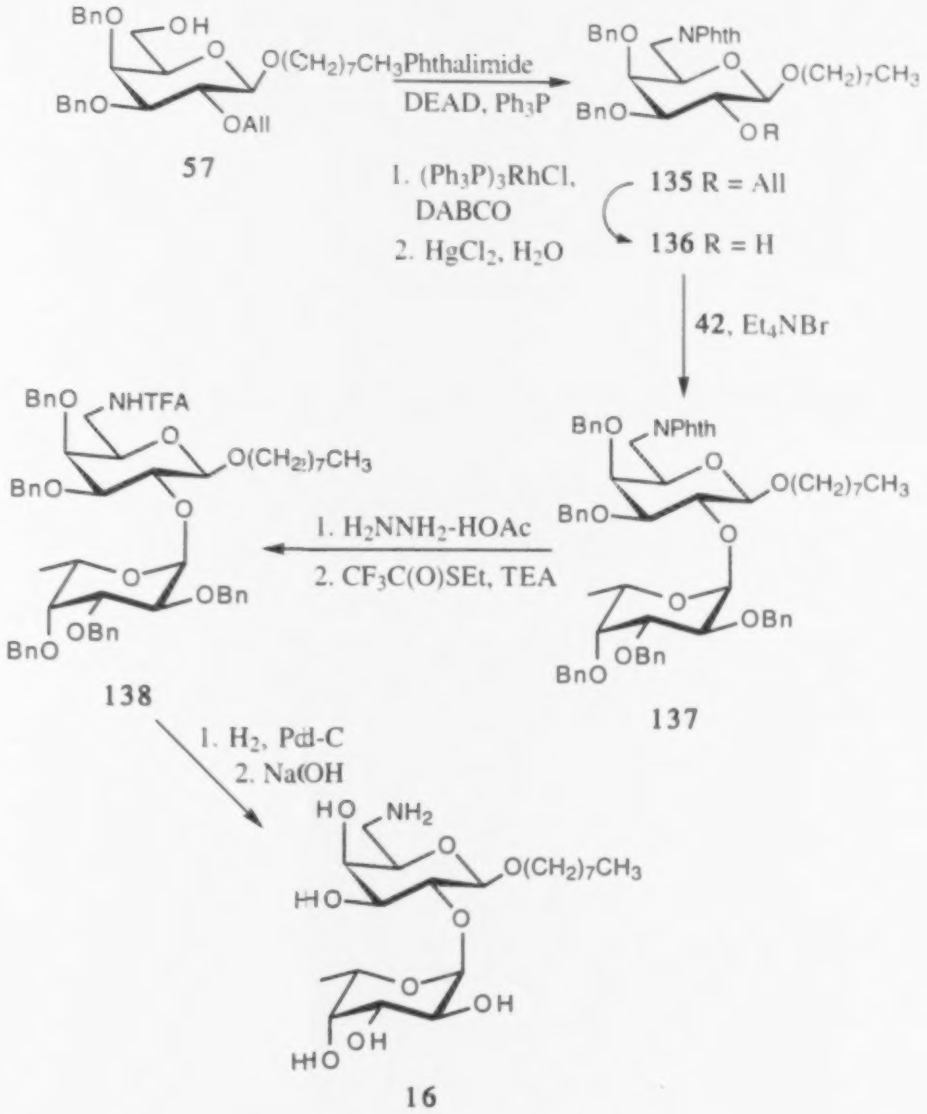

BnO
OH
O
O(CH2)7CH3
BnO
OAll
57
Phthalimide
DEAD, Ph3P
BnO
NPhth
O(CH2)7CH3
OR
135 R = All
136 R = H
1. (Ph3P)3RhCl, DABCO
2. HgCl2, H2O
42, Et4NBr
NPhth
OBn
137
1. H2NNH2-HOAc
2. CF3C(O)SEt, TEA
NHTFA
138
1. H2, Pd-C
2. NaOH
HO
NH2
OH
16

Fig 49. Preparation of the 6-amino disaccharide **16**.

followed by benozylation with dibutyltin oxide and benzoyl chloride (76%). Fucosylation of **133** was difficult, possibly due to steric interference from an axially disposed trifluoroacetate, and halide ion catalyzed fucosylation provided only small amounts of product (<5%). A more rigorous modification of the halide ion method, employing copper bromide[171], also gave similarly low yields of product. Finally, **134** could be obtained, albeit in low yield (42%), using silver triflate as the promoter. Compound **134** was deprotected by hydrogenation and hydrolysis to give the 4-amino disaccharide **15** (77%).

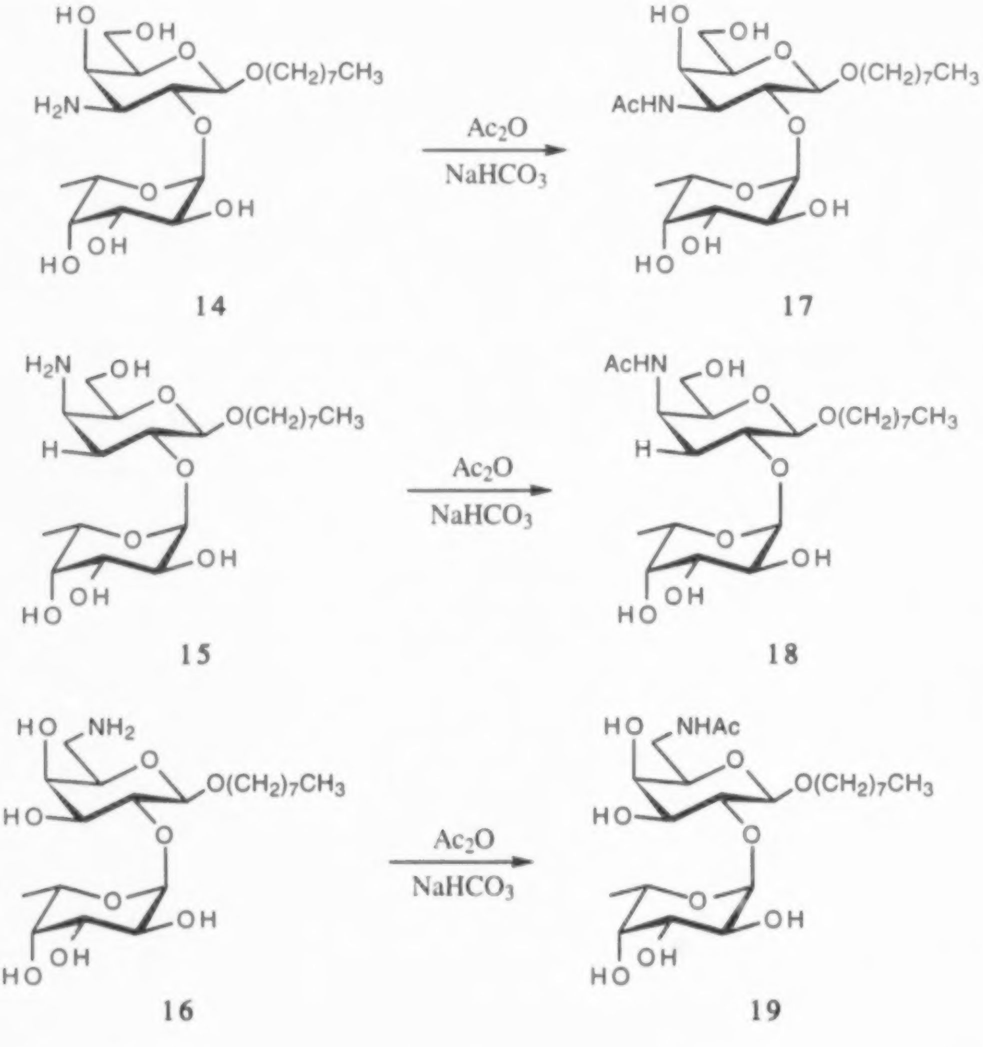
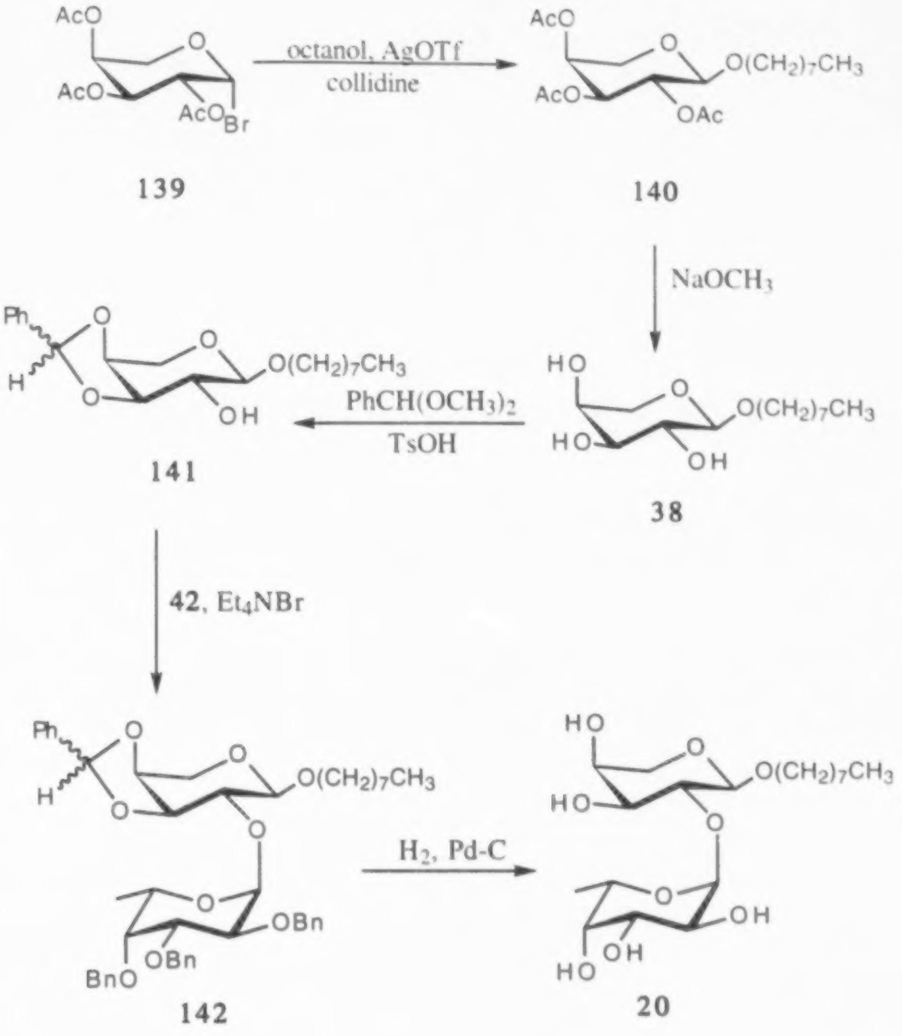

HO
OH
O
O(CH2)7CH3
H2N
O
O
OH
OH
HO
14
Ac2O
NaHCO3
AcHN
17
H2N
H
15
18
AcHN
HO
NH2
16
NHAc
19

Fig 50. Preparation of acetamido disaccharides **17-19**.



AcO
AcO
AcO
Br
octanol, AgOTf
collidine
AcO
AcO
OAc
O(CH$_2$)$_7$CH$_3$
139
140
NaOCH$_3$
Ph
H
O
O(CH$_2$)$_7$CH$_3$
OH
PhCH(OCH$_3$)$_2$
TsOH
HO
HO
OH
O(CH$_2$)$_7$CH$_3$
141
38
42, Et$_4$NBr
Ph
H
O(CH$_2$)$_7$CH$_3$
OBn
OBn
BnO
H$_2$, Pd-C
HO
HO
O(CH$_2$)$_7$CH$_3$
OH
OH
HO
142
20

Fig 51. Preparation of the*arabino* disaccharide **20.**

The preparation of the 6-amino derivative (Fig 49) involved first the conversion of alcohol **57**, by way of a Mitsunobu reaction[172] with phthalimide, to the 6-phthalimido derivative **135** (91%). Deallylation (65%) provided alcohol **136** which was fucosylated to give **137** (79%). The phthalimido group was removed and the amine protected as a trifluoroacetate (70%) and the compound then fully deprotected by hydrogenation and then saponification yielding **16** in 61% yield.

2. 2. 7 PREPARATION OF ACETAMIDO ANALOGS

The preparation of the acetamido derivatives was easily achieved by treating each of the disaccharide amines with acetic anhydride and sodium bicarbonate (Fig 50). Hence, **14** was acetylated to **17**, amine **15** provided **18** and the 6-amino disaccharide **16** converted to acetamide **19**.

2. 2. 8 PREPARATION OF ARABINO ANALOG

In order to prepare the C-5 dehydroxymethylated analog, the known bromide[173] **139** was prepared from L-arabinose. Conversion of **139** to the octyl glycoside proceeded in 69% yield and was followed by deacetylation to give the monosaccharide derivative **38** (90%). Protection of the 3,4 diol was achieved in 85% yield by the formation of a mixture of diastereomeric benzylidene derivatives **141**. The ratio of diastereomers was 4:1, the more polar isomer predominating. The major isomer was characterized and converted to disaccharide **142** by fucosylation with **42** (55%). The synthesis was completed by hydrogenation yielding **20** in 89% yield (Fig 51).

2. 2. 9 CONFORMATIONAL PROPERTIES OF ANALOGS

The conformational properties of disaccharides **2-20** have not been studied. However, from inspection of the ^{1}H NMR spectra of all the final products, the observed coupling constants of the anomeric protons were virtually identical to those observed in **2**, indicating that the individual monosaccharide rings are not significantly distorted.

2. 3 ENZYMATIC TESTING OF DISACCHARIDE ANALOGS

2. 3. 1 POTENTIAL SUBSTRATE ABILITY OF DISACCHARIDES

The activity of the synthetic disaccharide derivatives described above were determined using a radioactive assay technique which quantitated the rate of transfer of either ^{3}H-labeled Gal or GalNAc, from the corresponding commercial sugar nucleotides, to hydrophobic octyl acceptors as previously described[156]. Crude human serum was used as the source of the A- and B-transferases. After incubation, under were loaded onto SepPak cartridges and then washed with water and dilute ammonia until background counts (disintegrations per minute, DPM) were obtained. The radiolabelled reaction product was then eluted with methanol and quantitated by liquid scintillation counting. Kinetic plots are contained in the Appendix to this thesis.

Preliminary screening (Table 5) of compounds **2-20** as potential acceptors for the GalNAc and Gal transferases in human serum showed that in order for the analog to be a substrate leading to a stable product, the molecule should ideally possess first of all an intact 3,4 diol and secondly a substituent at C-6 which is similar in steric bulk and charge to the natural hydroxyl group. All analogs of **2** that were substrates had relative activities less than the native disaccharide, **2**. For those compounds possessing very low activities (e.g. the 3-fluoro analog), the counts obtained were within the experimental error of the experiment, and hence the analogs are effectively not acceptors. Those analogs listed in the table as having no activity, produced counts at or below background when assayed.

With the exception of the 3-epimeric (**12**) and 3-amino (**14**) analogs, the 3 modified derivatives are unable to react because of the lack of a suitable acceptor functionality at C-3. Both **12** and the **14** could potentially react, leading to a trisaccharide product. The epimer **12** could form a stable product, but it is clear from these results that the enzymes will not transfer to an axially oriented hydroxyl group at this position. The product formed from the amino analog **14** would have limited stability[176]. It is conceivable, therefore, that the amino derivative is in fact a substrate, but that the product is too labile to survive either in the assay mixture or the work-up procedure after the assay. That all of the Gal 4-OH derivatives are inactive points to that hydroxyl group being, according to the terminology of Lemieux, a key polar group[177] in the recognition by these enzymes.

Table 5. Relative acceptor activity of disaccharides **2-20** towards the blood group A (GalNAc) and B (Gal)-transferases in human serum.[a]

	% Activity	
Substrate	A-Transferase	B-Transferase
Native disaccharide **(2)**	100	100
3-Deoxy **(3)**	0	0
4-Deoxy **(4)**	0.1	0.2
6-Deoxy **(5)**	35	22
3-Fluoro **(6)**	0.3	0.1
4-Fluoro **(7)**	0	0
6-Fluoro **(8)**	43	30
3-O-Methyl **(9)**	0.4	0
4-O-Methyl **(10)**	0.3	0
6-O-Methyl **(11)**	13.4	3.2
3-Epimer **(12)**	0	0
4-Epimer **(13)**	0	0
3-Amino **(14)**	0.8	0.1
4-Amino **(15)**	0	0.3
6-Amino **(16)**	4.7	2.0
3-NHAc **(17)**	0	0
4-NHAc **(18)**	0.3	0
6-NHAc **(19)**	0.8	0
Ara **(20)**	2	0.3

[a] Compounds **2-20** were present at a concentration of 2.5 μM for the A-transferase and 10 μM for the B-transferase. Experiments were performed in at least duplicate with variation in replicates of less than 10%.

Table 6. Calculated kinetic constants[a] for acceptors **6**, **9** and **12** with the blood group A (GalNAc) and B (Gal)-transferases in human serum.

	Kinetic Constants			
	A-Transferase		B-Transferase	
Substrate	K_m[b]	V_{max}[c]	K_m[b]	V_{max}[d]
Native **(2)**	1.5 ± 0.2	0.61 ± 0.02	21.9 ± 3.4	0.32 ± 0.02
6-Deoxy **(5)**	7.3 ± 1.2	0.98 ± 0.07	68.8 ± 7.5	0.29 ± 0.02
6-Fluoro **(8)**	4.9 ± 0.8	0.45 ± 0.04	55.6 ± 9.5	0.31 ± 0.02
6-O-Methyl **(11)**	22.8 ± 3.0	0.58 ± 0.03	537.7 ± 18.8	0.36 ± 0.007
6-Amino **(16)**	74.5 ± 4.9	0.87 ± 0.02	565.3 ± 119.8	0.30 ± 0.003

[a] At saturating UDP-GalNAc and UDP-Gal concentrations

[b] K_m in μM

[c] V_{max} in pmol/min/0.61 μU A-transferase.

[c] V_{max} in pmol/min/0.32 μU B-transferase.

For the disaccharides modified at Gal OH-6, a broad spectrum of activities was observed. Both enzymes tolerate the sterically conservative substitution of the hydroxyl group with hydrogen (**5**) or fluorine (**8**). Both **5** and **8** remain substrates with reasonable activities; however, as the steric bulk of the group increases, the activity decreases. Thus the 6-O-methyl derivative (**11**) is a weak acceptor and the 6-acetamido (**19**) completely inactive. Interestingly, the smaller arabinose derivative (**20**) is practically inactive as well. Furthermore, although the replacement of the hydroxyl group with an amino functionality is more sterically conservative than hydroxy group methylation, the 6-amino analog (**16**) has a lower relative rate than **11**. It is also interesting to note that, although the enzymes mirror each other in terms of substrate specificity, the A-transferase appears to be more forgiving of substitutions at the 6-position. For all cases, when compared to **2** the relative activity of each analog with the A-transferase is higher than that for the B-transferase.

Determination of kinetic constants (Table 6) for the Gal OH-6 analogs **5**, **8**, **11** and **16** showed that the V_{max} is virtually constant for all analogs with the B-transferase. With the A-transfersase the V_{max} changes, but not dramatically, the highest rate being only twice the lowest. A comparison of the K_m values for these analogs shows significant differences between them with both enzymes. All compounds possess K_m's higher than the K_m of **2**. The best substrates, the fluoro (**5**) and deoxy (**8**) analogs, have K_m's of the same order of magnitude as that for **2** with both enzymes. The other less active analogs, **11** and **16**, possess K_m's an order of magnitude higher than the K_m of **2**.

2. 3. 2 POTENTIAL INHIBITOR ABILITY OF DISACCHARIDES

Results from the evaluation of disaccharides modified at the 3 and 4 positions as potential inhibitors are presented in Table 7. As before, all the derivatives with modifications at the 4-position were inactive, demonstrating again that the galactosyl 4-hydroxyl group is crucial for binding to the enzyme. All of the Gal OH-3 analogs showed some inhibitory activity with both enzymes. The levels of inhibition ranged from almost complete inhibition by the 3-amino compound, **14** to almost no inhibition by the 3-O-methyl derivative, **9**.

Although, as mentioned above, the A-transferase appears to be more forgiving of replacement of the 6-OH, it appears that the converse is true of analogs with modifications at Gal OH-3. This is true especially of groups with more sterically demanding modifications. For example, the 3-acetamido derivative **17**, shows dramatic differences in the amount of inhibition observed between the two enzymes. With the A-transferase almost no inhibition is seen while for the B-transferase **17** is a fairly good inhibitor. A similar, though less pronounced, effect is seen with both the O-methyl

Table 7. Relative inhibitor ability of Gal OH-3 and OH-4 modified disaccharides towards the blood group A (GalNAc) and B (Gal)-transferases in human serum.

	% Inhibition	
Substrate	A-Transferase[a]	B-transferase[b]
3-Deoxy **(3)**	30	85
4-Deoxy **(4)**	2	0
3-Fluoro **(6)**	22	24
4-Fluoro **(7)**	0	0
3-O-Methyl **(9)**	4	15
4-O-Methyl **(10)**	0	2.5
3-Epimer **(12)**	36	88
4-Epimer **(13)**	0	0
3-Amino **(13)**	98	93
4-Amino **(15)**	0	1.8
3-NHAc **(17)**	7.1	52
4-NHAc **(18)**	0	0
6-NHAc **(19)**	0	0
Ara **(20)**	0	0

[a] Concentration of potential inhibitor was 25 μM with parent acceptor at 2.5 μM.
[b] Concentration of potential inhibitor was 100 μM with parent acceptor at 10 μM.

analog **9** and the epimeric derivative **14**. For those analogs with modifications designed to probe hydrogen bonding interactions, the effect is not observed. The deoxy compound **3** is an exception, however, as a significantly higher inhibition is seen with the B-transferase.

TABLE 8

Calculated inhibition constants (K_i)[a] of Gal OH-3 modified disaccharides with the blood group A and B glycosyltransferases in human serum.

	A-transferase		B-transferase	
Substrate	Ki (µM)	inhibition mode	Ki (µM)	inhibition mode
3-Deoxy **(3)**	68.3 ± 9.4	competitive	14.0 ± 1.5	competitive
3-Fluoro **(6)**	48.0 ± 9.3	competitive	110.5 ± 15.6	competitive
3-Methyl **(9)**	313[a]	n.d.[b]	283[a]	n.d.
3-Epimer **(12)**	22[a]	complex	7.8 ± 0.8	competitive
3-Amino **(14)**	0.2[a]	complex	5[a]	complex
3-NHAc **(17)**	200[a]	n.d.	46[a]	n.d.[b]

[a] Estimated $\underline{K}_i$ assuming the inhibition is competitive and calculated from the equation $\underline{i} = [\underline{I}]/([\underline{I}] + \underline{K}_i\{1 + [\underline{S}]/\underline{K}_m\})$, where $\underline{i}$ is the fractional inhibition, [I] the inhibitor concentration, and [$\underline{S}$] the substrate concentration.[175]

[b] Not determined

Table 8 shows the calculated or estimated inhibition constants (K_i's) for inhibitors **3**, **6**, **9**, **12**, **14** and **17**, which were in the range of 200 nM to 313 µM. Of special interest is the estimated K_i of 200 nM for the 3-amino compound **14** with the A-transferase, which is the first reported example of a sub-micromolar glycosyltransferase inhibitor. For compounds that were weak inhibitors of one or both enzymes (the 3-O-methyl and 3-acetamido analogs, **9** and **17**), no effort was made to experimentally determine exact K_i's. Instead an estimated K_i was calculated[175] using the formula shown in the legend for Table 8. This equation is based on the assumption that the inhibition is competitive. The percent inhibition used (i) was the value found in Table 7 for the inhibitor at roughly 10 times the K_m for the A-transferase and 5 times the K_m for the B-transferase. A comparison of these K_i values with the K_m of **2** provides a useful qualitative insight into what groups the enzymes require. A quantitative comparison is not meaningful for enzymes such as these which likely proceed though a two step mechanism since K_m values need not represent true dissociation constants.

The deoxy (**3**) and fluoro (**6**) analogs were shown to be competitive inhibitors of both enzymes. The K_i's of both with the A-transferases are significantly (greater than 30 fold) higher than the K_m of **2**. As mentioned above, the B-transferase appears to recognize these analogs with greater affinity since the K_i of the deoxy analog **3** is less than the value for the K_m of **2**. As well the K_i of the fluoro analog is roughly only five fold higher than the K_m of **2**, as compared to 30 fold higher for the A-transferase. The 3-epimer **12** was determined to be a competitive inhibitor of the B-transferase, with a K_i below that of the K_m for **2**. The 3-amino derivative (**14**) was tested as an inhibitor of the B-transferase, but the observed inhibition kinetics did not follow a simple mechanism. Concomitant with an increase in inhibitor concentration, a pronounced downward curvature was observed (data not shown) in the inverse plots used to determine the K_i. The magnitude of the curvature increased with the inhibitor concentration. As well, attempts to determine the K_i and mode of inhibition for **12** and

14 with the A-transferase were not successful. As in the case of the inhibition of the B-transferase by **14**, the same downward curvature was observed in the inverse plots.

Similar results were obtained with **14** when tested with A-transferase serum from a different donor indicating that the effect is not related to the serum donor. Rather than evaluating the complex pathway by which these enzymes inhibit these enzymes using crude serum, we decided to wait until we had purified enzymes to do the detailed kinetic studies. Both enzymes have been recently cloned by a collaborator (D. Bundle) and once they are expressed, the determination of the inhibition mechanism can be determined much more easily.

Although, we have been unable to show that this amino derivative is a competitive inhibitor, we feel that the mechanism is, at least to some degree, competitive. In support of this contention, we tested, as a control, the monosaccharide precursor to **14**, octyl 3-amino-3-deoxy-β-D-galactopyranoside, and found it to be inactive as an inhibitor. We believe that this result, along with the fact that neither the 4-amino or 6-amino derivatives inhibit the enzyme, support a defined recognition of the inhibitor by the enzyme. A defined recognition mechanism would rule out inhibition resulting from another effect such as pH or the binding of the inhibitor to another part of the enzyme and altering its conformation, thus decreasing the enzyme's activity.

Before two possible inhibition mechanisms for the 3-amino compound are presented, a brief digression is necessary to discuss what is known about the order in which the substrates are bound to the enzyme. It is not known definitively whether the acceptor or donor binds first to these enzymes, or if the binding is random. There is, however, some indirect evidence that the donor either binds to the enzyme first, or at least that the binding of the donor is not dependent on the acceptor being bound. This information comes from an analysis of the methods routinely used to purify these enzymes. First of all, it is known that glycosyltransferases can be purified by binding them to affinity columns made from their donor precursors[178]. For the A and B-transferases, a solution of the enzyme would be passed over a column containing bound UDP, to which the enzyme binds. Removal of enzyme is then achieved by washing the column with UDP. The ability to do this suggests that the presence of the acceptor is not required for the donor to bind. Additional information comes from a paper on the purification of the B-transferase. In this report[179], Watkins and coworkers used O-erythrocytes as an acceptor-based "affinity column" to bind the enzyme. The enzyme would not bind to the erythrocytes unless UDP-Gal was present, which suggests that in order for the acceptor to bind, the donor must be bound. Both of these observations strongly point to the donor binding first.

In the introduction, a two-step displacement mechanism involving a glycosyl-enzyme intermediate was suggested for these transfers. Assuming, for the sake of argument, that this is indeed true, a key question that remains is whether the binding of the acceptor precedes or follows the formation of this intermediate. No work has been published addressing this issue. For the discussion that follows, to provide a basis for rationalizing the results, I will assume that the formation of this intermediate follows the binding of both the donor and acceptor molecules (Fig 52).

Returning now to the possible inhibition mechanism, the simplest explanation for inhibition by **14**, is that the amine inhibits by binding tightly to the enzyme and precludes binding of either the donor, the acceptor or both (Fig 53). If this were true, the compound should behave as a competitive inhibitor. The observation that the inhibition is complex suggests that the mechanism is not that simple.

A second, more tenuous explanation, involves first the binding of the donor to the enzyme. The acceptor then binds and the formation of the glycosyl enzyme intermediate and UDP follows (Fig 54). The liberated UDP possesses two negative charges, one of which could deprotonate the amine. The formation of a deprotonated amine in the active site might lead to the formation of an amino-linked trisaccharide. Such a product, as mentioned above, would not be stable and the product would

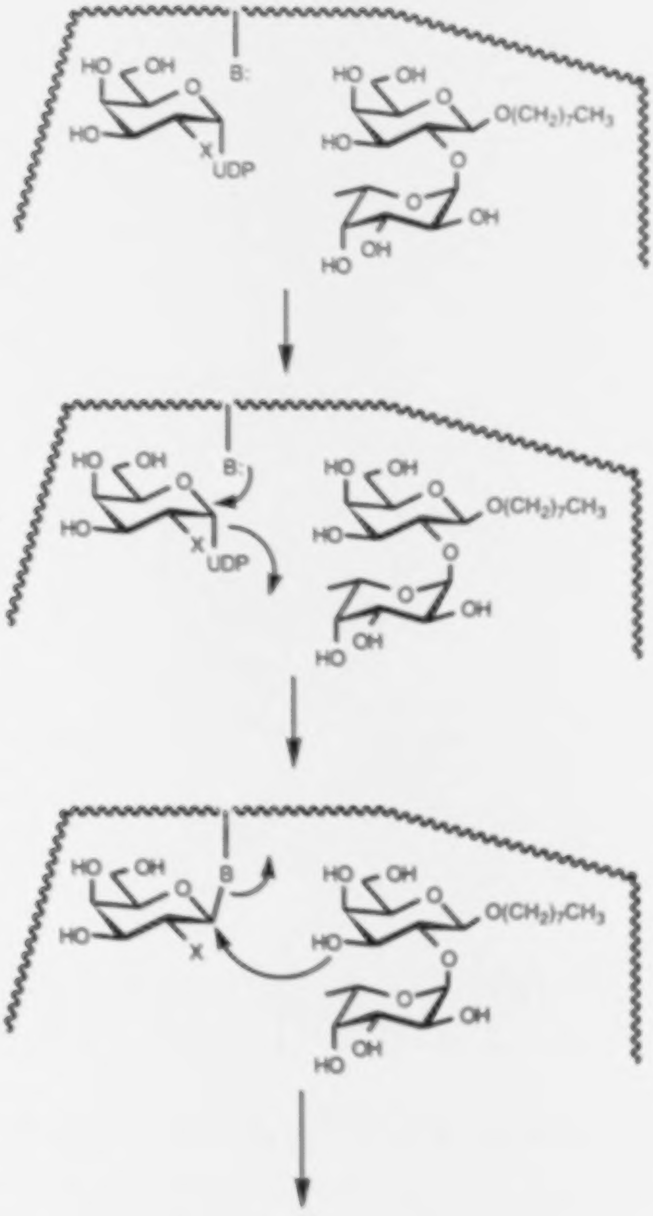

HO
OH
O
HO
X
UDP
B:
O(CH2)7CH3
OH
B
Product

Fig 52. Proposed binding mode where both substrates bind before the formation of the glycosyl-enzyme intermediate.

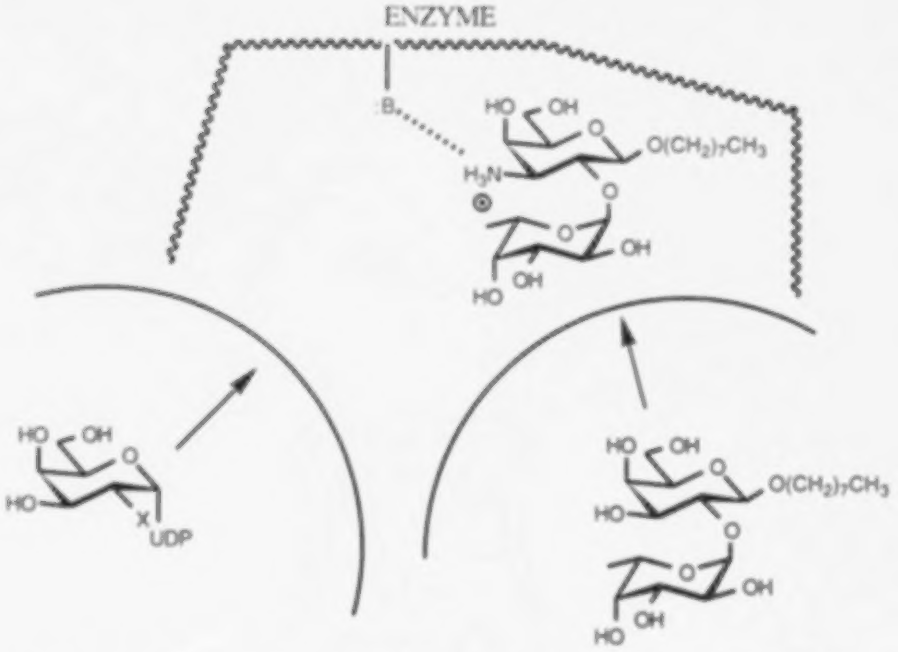


Fig 53. Simple competitive inhibition by the 3-amino disaccharide **14**.

hydrolyze. The hydrolysis could occur either in the active site or after the product dissociated from the enzyme. However, the inhibitor must remain in close association with enzyme in order to explain the large amounts of inhibition observed. In effect the inhibitor would be acting to hydrolyze the sugar nucleotide donor. This mechanism depends on a number of factors including the close association of the inhibitor with the active site. At the same time the binding of the compound must be such that the sugar nucleotide can bind, be hydrolyzed and then leave, allowing another molecule to bind. As pure cloned enzyme becomes available, the mechanism in Fig 54 can be assessed by looking for UDP production in the presence of **14**.

In summary of the results presented, it appears, that the both the A and the B-transferases will tolerate some modifications at the galactosyl 3 and 6-hydroxyl groups without loss of acceptor recognition. However, those analogs which differ widely from the natural substrate are either recognized poorly or not at all. Furthermore, substitution

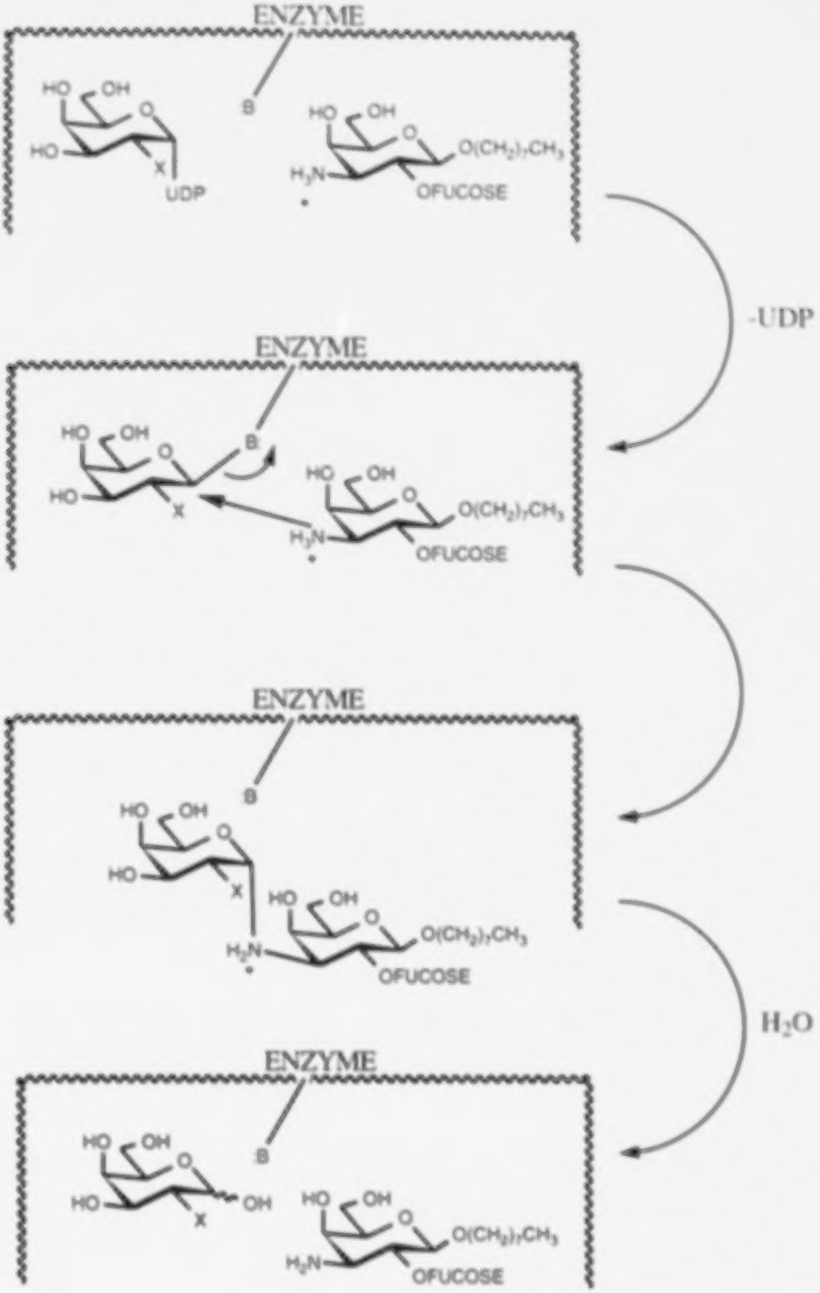

ENZYME
B
HO
OH
O
X
UDP
H₂N
OFUCOSE
O(CH₂)₇CH₃
-UDP
H₂O

Fig 54. Suggested complex inhibition mechanism by the 3-amino disaccharide **14.** X = OH or NHAc.

of the galactosyl 4-hydroxyl group with any of the functional groups used in this study precludes binding; that is, this hydroxyl group is involved in a key polar interaction[177] with the enzyme.

2. 4 ANALYSIS OF EXPERIMENTAL RESULTS

In the absence of either X-ray crystallographic data or the knowledge of where the active site is, any attempt to outline the important carbohydrate-protein interactions in the active site with any certainty is difficult at best. Additionally, detailing the important active site interactions is also hampered by a lack of information about the conformational properties of these analogs. Nevertheless, some hypotheses can be put forth. In the remainder of this chapter, I will discuss some possible features that might be present in the active site based on the results obtained with these synthetic disaccharides. Admittedly, while the model described below is highly speculative, it will hopefully provide a basis for future explorations into the critical binding interactions in these active sites.

2. 4. 1 INTERACTIONS AROUND GAL OH-6

The observed changes in acceptor ability: 6-OH > 6-fluoro > 6-deoxy > 6-O-methyl > 6-acetamido could easily be explained by the presence of an amino acid residue that precludes binding of larger analogs thorough an unfavorable steric interaction. However, from this model, it would be predicted that both the and 6-amino and *arabino* derivatives should also be recognized reasonably well by these enzymes since these groups are, respectively, nearly the same size or smaller than the native hydroxyl group. It could be argued, as well, that the protonated amine is extensively hydrated in solution, and that this hydrated complex presents a group too sterically demanding for the enzyme to easily bind. The fact that the *arabino* derivative is not active is more difficult to explain, but could suggest an important interaction between the C-6 methylene group and the enzyme.

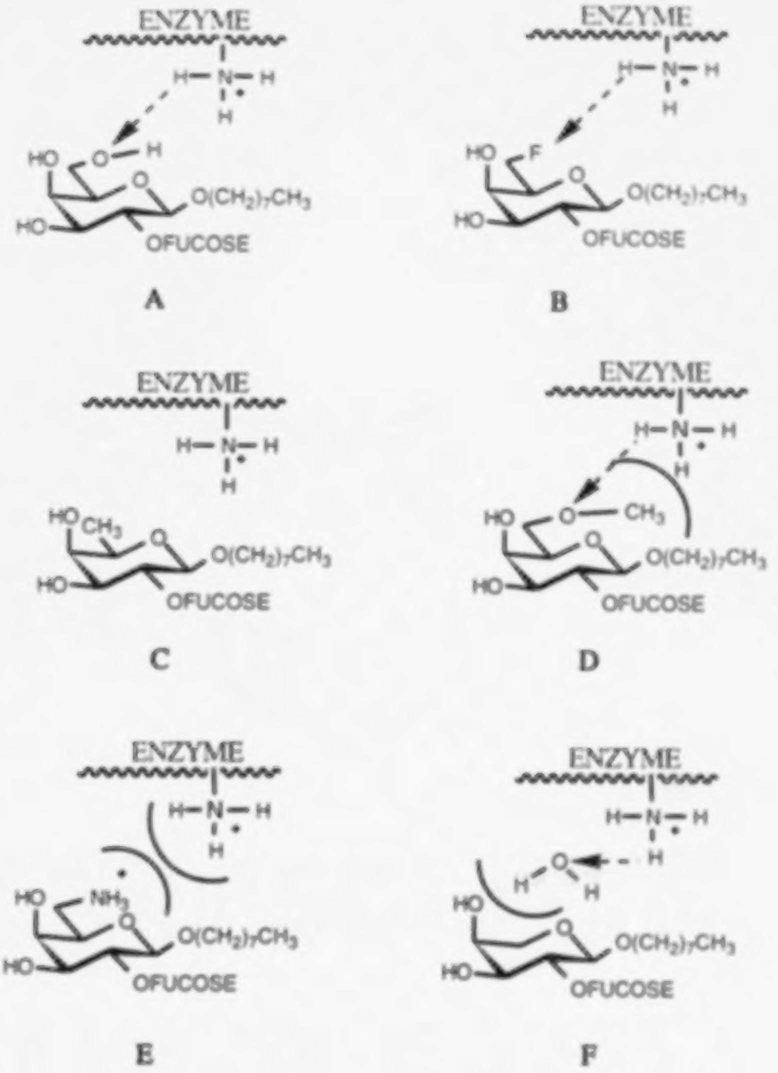

ENZYME
H—N—H
H
HO
O—H
F
CH3
O—CH3
NH3
H—O—H
O
O(CH2)7CH3
HO
OFUCOSE
A
B
C
D
E
F

Fig 55. Proposed active site interactions near Gal OH-6.

Another, perhaps more attractive, hypothesis is that there is, in the vicinity of Gal OH-6, a positively charged amino acid residue (e.g. a protonated lysine), that also prohibits recognition of sterically demanding substrates (Fig 55 A). A sterically inhibitory and positively charged polar environment at this position could also explain the observed results as follows. The fluoro derivative would remain a good substrate, due to its highly electronegative character, and its ability to accept a hydrogen bond from the positively charged group (Fig 55 B). With the deoxy derivative, the ability to hydrogen bond is now lost and additionally a nonpolar group is placed into the polar environment, thus destablizing the complex. Due to its small size, however, it is still recognized by the enzyme (Fig 55 C). The O-methyl derivative is both sterically demanding and also places a hydrophobic group near this positively charged residue in the active site, and thus the complex is destablized much more so than with the 6-deoxy compound (Fig 55 D). When the size of the group is increased further, to the acetamido derivative, recognition is lost altogether through steric hindrance. With the positively charged, protonated amino group at C-6, the complex is again destablized by a strong electrostatic repulsion of two positively charged groups in that area (Fig 55 E). Alternatively, as mentioned above, the protonated amine group, with a shell of hydration from the solvent, could provide the enzyme with a substrate too bulky to bind, thus destabilizing the complex through steric hindrance. However, the fact that the 3-amino derivative is a potent inhbitor of both enzymes suggests that the hydration of the amine may not be important. Finally, to explain the lack of activity of the *arabino* derivative, the shuffling of water molecules, as reported in some of Quiocho's studies[147, 150], could be important. The active site, prior to binding, has a shell of water along its surface. In order to bind the substrate this hydration shell must be expelled to bulk solvent. It is possible that, without a group larger than hydrogen at C-5 of L-arabinose, the driving force to expel a water molecule at that area of the active site is not present (Fig 55 F). Through hydrogen bonds, a positive charge in this area would hold such a water molecule in place. In other words, a molecule of water remains in the active site, and thus sterically precludes binding of this substrate by the enzyme.

The previously mentioned pattern of the A-transferase being more tolerant of substitution at the 6 position than the B-transferase could be rationalized by using the computer modeling studies of Hakomori, mentioned in the introduction[120]. In those studies, the amino acid differences between the A- and B-transferases were shown to decrease the flexibility of the B-enzyme relative to the A-enzyme. Thus the A-tranferase, being more flexible, could alter its conformation more readily, accepting unnatural analogs with greater ease.

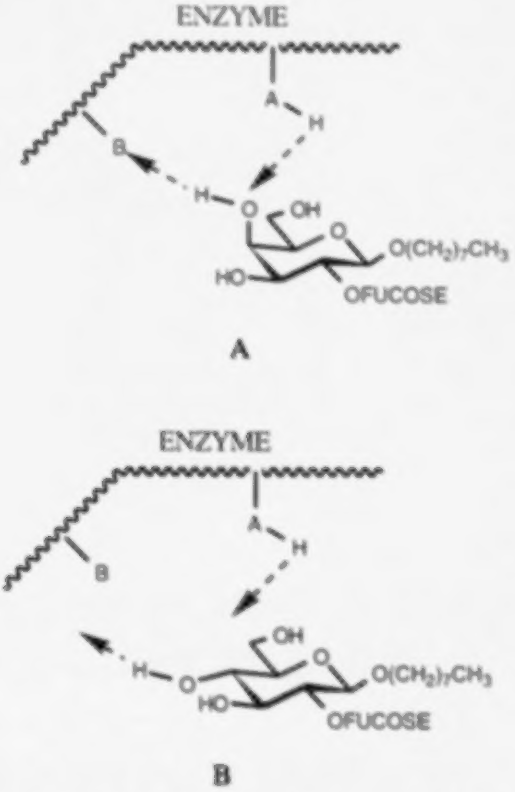


Fig 56. Proposed active site interactions near Gal OH-4.

2.4.2 INTERACTIONS AROUND GAL OH-4

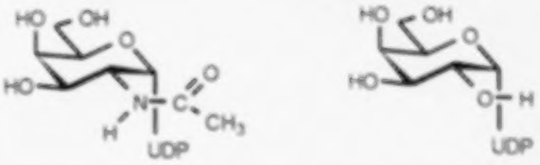
Since all the analogs at this position are inactive, it appears that this hydroxyl group is involved both in accepting and in donating hydrogen bonds (Fig 56 A). Of all the analogs, only the acetamido derivative and the *gluco* derivative can fulfill both of these requirements. In the acetamido case, the lack of activity can easily be ascribed to an unfavorable steric interaction. The inactivity of the *gluco* isomer suggests that, compared with *galacto* isomer, the distance between the 4-OH group and the important active site amino acid residues is probably too great (Fig 56 B). It is also possible that, when equatorial, the hydroxyl group at C-4 interacts in a sterically unfavorable manner with the enzyme.

2. 4. 3 INTERACTIONS AROUND GAL OH-3

The most exciting information obtained from this study is the extremely potent inhibition of both transferases by the 3-amino compound, **14**. That this compound is an inhibitor of both enzymes supports our hypothesis of the possible existence of a negatively charged residue in the vicinity of the Gal OH-3 in the acceptor-binding site of these transferases. Since carboxylates are present in all of the crystal structures mentioned in the introduction, it seems quite possible that this negatively charged amino acid residue is either an asparate or glutamate residue. Using the presence of an active site carboxylate as a basis, the inhibition effects seen with the other analogs can be rationalized in the following model.

As mentioned earlier in the discussion, those compounds that were intended to probe steric interactions at Gal OH-3 (the 3-O-methyl, 3-acetamido and 3-epimeric analogs) all showed significantly higher amounts of relative inhibition with the B-transferase than with the A-transferase. Of the remaining analogs, intended to probe hydrogen bonding requirements, two (the 3-amino and 3-fluoro) were shown to have fairly similar effects in terms of relative inhibition with both enzymes. The other, the 3-deoxy compound, followed a pattern similar to the "steric-probing" analogs.

Since Gal OH-3 is the site of reactivity, it is important to consider not only the acceptors, but also the donors in both reactions. It should also be recalled, that for the purposes of this discussion I have assumed that the formation of the glycosyl-enzyme intermediate follows the binding of both donor and acceptor to the enzyme. The donors for both reactions are identical in every respect except the size of the group at C-2, the



UDP-GalNAc UDP-Gal

Fig 57. Comparison of the steric bulk of the substituent at C-2 in UDP-GalNAc and UDP-Gal.

carbon adjacent to the site of reactivity (Fig 57). The donor for the A-transferase, UDP-GalNAc has a bulky acetamido group at C-2, while the B-transferase donor, UDP-Gal, has a much smaller hydroxyl group at C-2. Due to the close proximity of these structural differences in relation to the reaction site they are likely to affect the binding of the acceptor.

In order to explain the ability of the B-transferase to recognize analogs with groups of larger steric bulk at C-3 (3-O-methyl and 3-acetamido) it can be hypothesized that because the B-donor presents less steric congestion then the A-donor, there is more room available the binding of larger groups. The same explanation can be used for the preferred binding of the 3-epimer by the B-transferase (Fig 58).

The 3-fluoro and 3-amino compounds do not show differential inhibition between the two enzymes. Of all the analogs, these two, that are directed towards probing for hydrogen bonding interactions are the most sterically similar to the native structure. It could therefore be expected that they would bind in a manner similar to the parent disaccharide. We believe that the amino compound, as mentioned above, might be a tight binding inhibitor because of an ionic interaction between the protonated amine and the negatively charged carboxylate residue (Fig 59 A). This carboxylate could also play a role in the relatively weak inhibition by the fluoro analog. In the introduction to this thesis, work of Quiocho was cited which showed that the repulsion between an

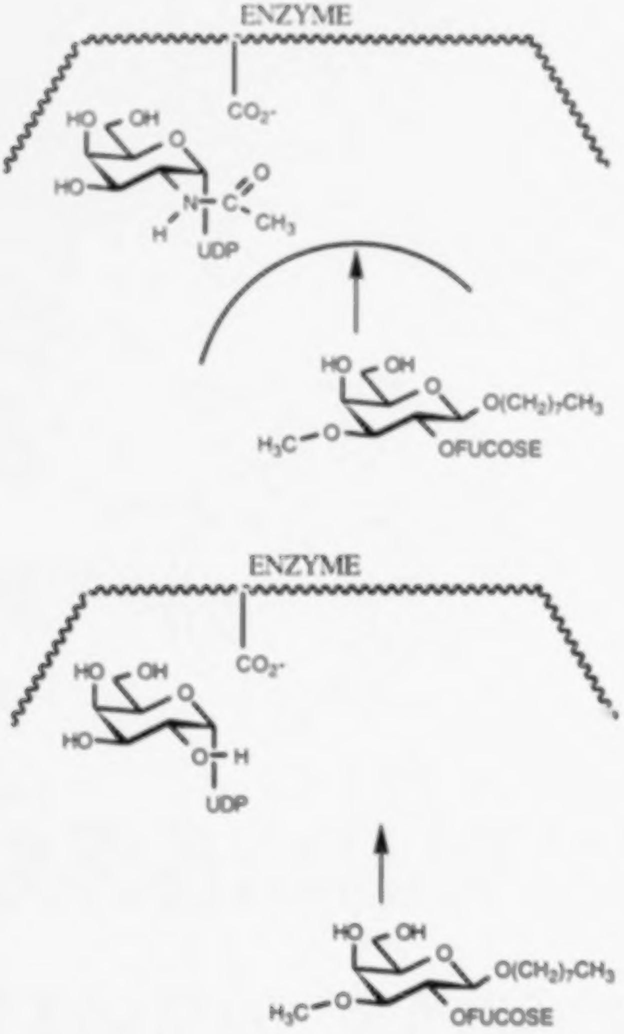

ENZYME
CO2-
HO
OH
O
HO
N
C
O
CH3
H
UDP
HO
OH
O
O(CH2)7CH3
H3C—O
OFUCOSE
ENZYME
CO2-
HO
OH
O
HO
O
H
UDP
HO
OH
O
O(CH2)7CH3
H3C—O
OFUCOSE

Fig 58. Comparison of the A and B-transferases' ability to recognize groups of larger steric bulk at C-3, using the 3-O-methyl analog as an example.

electronegative fluorine and an active site aspartate resulted in weak binding of a fluorinated analog of the naturally binding substrate[150]. The case here is identical. An electrostatic repulsion between the catalytically active carboxylate and the fluorine at C-3 would result in the observed weak binding by this inhibitor (Fig 59 B).

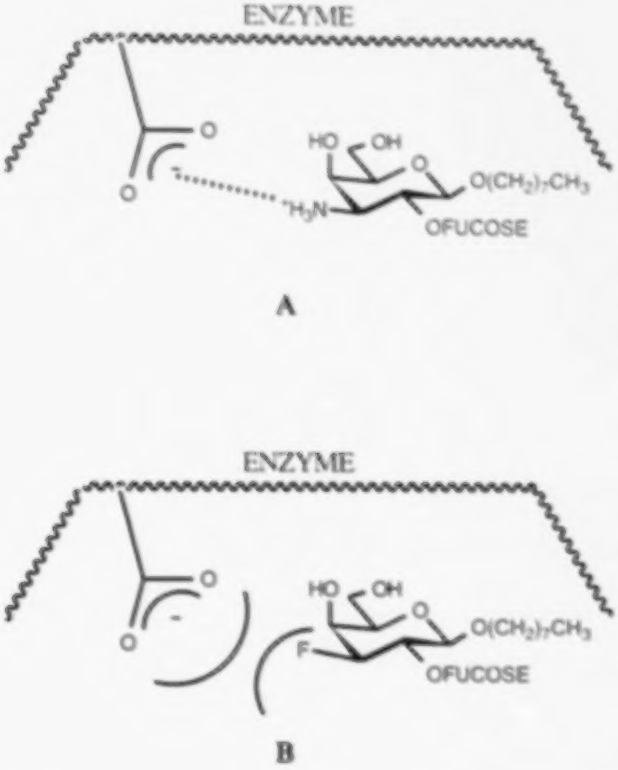
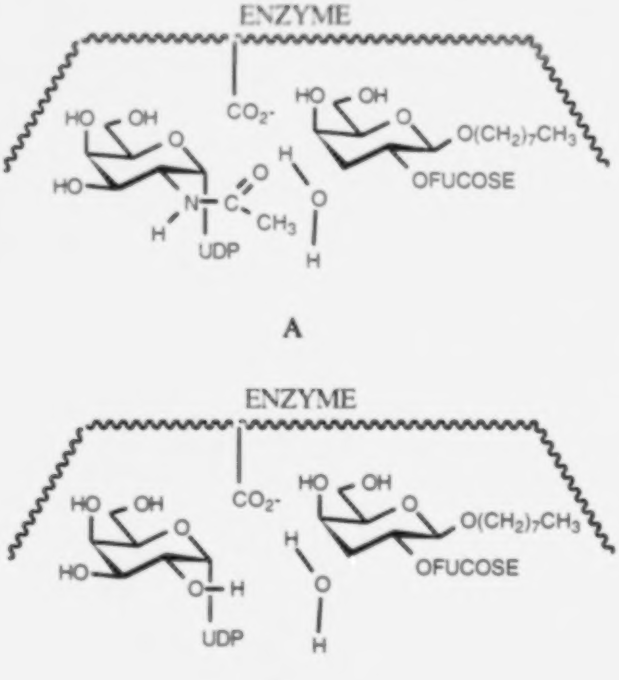


Fig 59. Comparison of binding of 3-amino and 3-fluoro disaccharides.

Finally, the 3-deoxy analog shows different degrees of inhibition with each enzyme. This analog has group smaller in steric bulk than the native structure. To explain this effect, both the size of the donor and the presence of a water molecule in the active site could be important. For both enzymes, it is possible that with the smaller deoxy analog, a water molecule can remain in the active site, filling the gap left by the hydroxyl group. With the A-transferase, the presence of the C-2 acetamido group creates a bad steric interaction with this water molecule, thus the compound binds weakly to the enzyme (Fig 60 A). The more spacious active site in the B-transferase would accommodate the water molecule more easily and therefore the deoxy compound would be a better of the B-transferase than of the A-transferase (Fig 60 B).



A

B

Fig 60. Comparison of the A and B-transferases' ability to recognize the 3-deoxy disaccharide.

In conclusion, I again stress that the model presented above is highly speculative. It will be interesting to see, as the cloned enzyme becomes available and more detailed studies can be done, which, if any, of these hypotheses are true. Along these same lines, it appears that two of our initial hypotheses regarding the binding of these substrates may be incorrect. Two pieces of information rule out the potential for a bidentate hydrogen bond between the galactosyl 3,4-diol and an enzymatic carboxylate or amide group, as described for other galactose recognizing proteins. The ability of the enzymes to recognize analogs modified at OH-3 of the galactosyl residue coupled with the observation that the 4-amino derivative is not inhibitory weigh heavily against this hypothesis. Secondly, the fact that the 3-epimer inhibits both enzymes suggests that a hydrophobic interaction, via van der Waals interactions, from the bottom side of the Gal residue is not present. Such an interaction would be greatly disturbed by the axially oriented hydroxyl group in the 3-epimer.

2. 5 SUGGESTIONS FOR FUTURE WORK

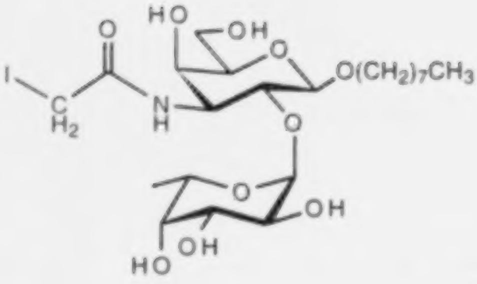
These results point to some obvious directions to head in the future to further probe important active site interactions. I will divide these into two areas: 1.) chemical synthesis and evaluation of new analogs and 2.) enzymatic studies with existing analogs.

2. 5. 1 CHEMICAL SYNTHESIS AND EVALUATION OF NEW ANALOGS

In terms of ease of preparation, the 3-iodoacetamido disaccharide (**143**) analog would be the most readily prepared derivative. Its formation requires only one chemical step from the already synthesized amine. Since the B-transferase will recognize the 3-acetamido derivative as an inhibitor, this iodoacetamide might also be recognized by the enzyme. If a catalytic active base is in the active site, this compound could serve as a covalent irreversible inhibitor. Displacement of the iodine by the reactive base will therefore result in deactivation of the enzyme. The epoxide **144** or aziridine **145** could also serve as irreversible inhibitors, although their chemical synthesis would be much more challenging.

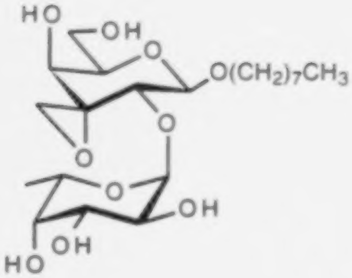
Also of potential interest in mapping out the interactions near C-6 would be the galacturonic acid derivative, **146**. If a positively charged active site residue is in the vicinity of this carboxylate, tight binding would be expected. This would give further insight as to whether there is positive charge in that area, or whether the observed pattern of recognition is due only to steric interactions.

The diazirene, **147**, or azide, **148**, could also serve as deactivating analogs. When photolyzed, the compounds would generate, respectively, a carbene or nitrene. These reactive intermediates would then insert into the protein, allowing the labeling of the active site with an acceptor substrate. Once the cloned enzymes are available, these



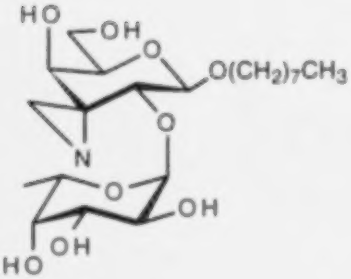
O
I
C
H2
N
H
HO
OH
O
O(CH2)7CH3
O
O
OH
OH
HO

143



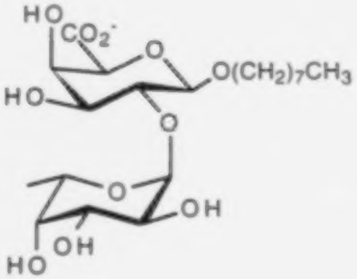
HO
OH
O
O(CH2)7CH3
O
O
O
OH
OH
HO

144



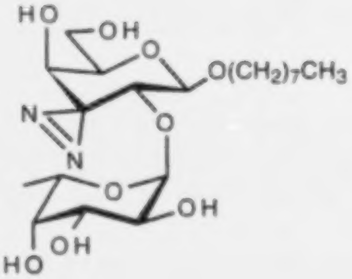
HO
OH
O
O(CH2)7CH3
O
N
O
OH
OH
HO

145



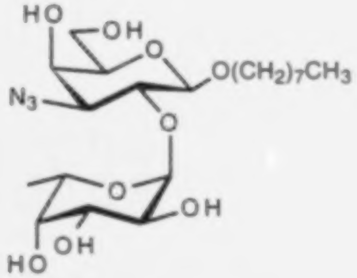
HO
CO2-
O
O(CH2)7CH3
HO
O
O
OH
OH
HO

146



HO
OH
O
O(CH2)7CH3
N
N
O
O
OH
OH
HO

147



HO
OH
O
O(CH2)7CH3
N3
O
O
OH
OH
HO

148

Fig 61. Suggested disaccharide analogs for further probing of the active sites of A and B-transferases.

compounds would be useful for determining the position of the active site via tagging the protein with a radiolabelled precursor, followed by amino acid sequencing.

Finally, a study similar to this work, where the fucose moiety has been systematically modified would provide preliminary data on the important groups in that portion of the molecule.

2. 5. 2 ENZYMATIC STUDIES WITH EXISTING ANALOGS

The most pressing task to be completed is the determination of the exact mechanism by which the amino and epimeric compounds inhibit the enzyme. As mentioned above this would be best done on purified enzymes.

Additionally, it should be determined, unequivocally, the order in which the donor and acceptor bind. Also important would be the point at which the proposed glycosyl-enzyme intermediate forms.

Testing these analogs with serum from a *cis*-AB[104] individual would also be interesting. Since some of the compounds exhibit differential recognition between the two transferases, it would be interesting to see what the recognition would be with an enzyme that uses either sugar nucleotide. Also, testing these with an AB individual who is not cis-AB might allow the inhibition of one enzyme over the other. The ability to inhibit the formation of one of the two antigens selectively could be of importance in transplant operations and blood transfusions.

Finally, once the cloned enzymes are obtained in significant quantities, the availability of this wide panel of substrates, would enable a very thorough study of the binding. For example, the more meaningful binding constants instead of K_m's, could be determined using microcalorimetry[180]. Also, with a significant amount of the enzyme, an effort could be made to obtain crystal structures of a number of the analogs bound to the enzymes.

CHAPTER THREE:

RECOGNITION OF SYNTHETIC MONOSACCHARIDE ANALOGS OF THE ACCEPTOR β-D-Gal*p*-OR BY THE H-GLYCOSYLTRANSFERASE

3. 1 INTRODUCTION

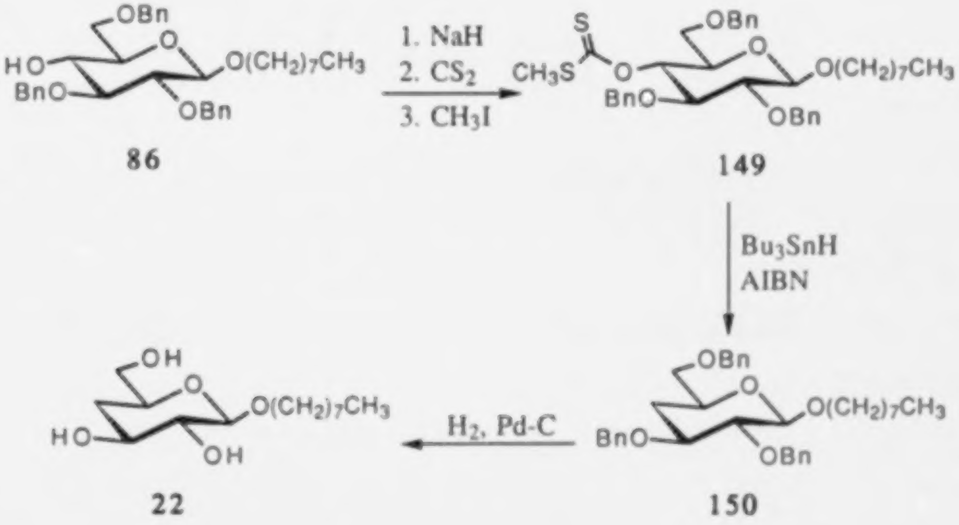
The chemical synthesis and enzymatic testing of the monosaccharide derivatives **21**-**38** are discussed in this chapter. Compared to the A and B-transferases, this α (1→2) fucosyltransferase (H-transferase) shows a less clear-cut acceptor specificity pattern. This is probably due in part to the fact that although octyl β-D-galactopyranoside is a substrate, it is a weak binding one with a K_m in the millimolar range. Much stronger recognition is achieved with the Type I-Type VI disaccharide structures discussed in the introduction. We anticipated that, as a result of the relatively weak binding of 1, the monosaccharide analogs synthesized would have K_i's too high to be useful inhibitors. Therefore we decided to determine only relative acceptor and inhibitor activities and not to carry out the more detailed kinetic calculations described in Chapter 2. Nevertheless, we felt that this initial probing study would be best done at the monosaccharide level where the analogs could be easily prepared. Probing the H-transferase substrate specificity with disaccharide analogs would have been a much larger undertaking, The information presented here will be useful in that it can be used as a starting point to make analogs of the Type 1-Type VI structures. The K_m values for the Type I disaccharide with an α (1→2) fucosyltransferase from porcine submaxillary gland, has a K_m of 200 μM[71, 181]. Therefore, inhibitors based on this structure should be more useful than the monosaccharide analogs.

3. 2 CHEMICAL SYNTHESIS

The chemical synthesis of monosaccharides **21**-**38** was achieved, for the most part, in the course of preparing the disaccharides required for the A and B-transferases. The preparation of the following derivatives was discussed in Chapter 2: 3-deoxy (**21**), 3-fluoro (**24**), 4-fluoro (**25**), 6-fluoro (**26**), 3-O-methyl (**27**), 3-epimer (**30**), 4-amino (**33**), and *arabino* (**38**). The 4-epimer, octyl β-D-glucopyranoside, is commercially available and was purchased from Sigma Chemical Company, St. Louis MO. The preparation of the remaining analogs is discussed below.

3. 2. 1 PREPARATION OF DEOXY ANALOGS

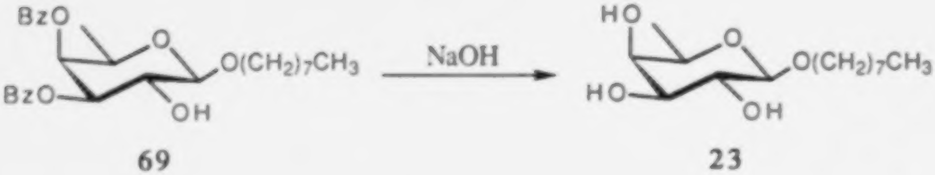
The 4-deoxy derivative was prepared in three steps from alcohol **86** (Fig 62). Conversion of this alcohol to the 4-xanthate (**149**, 61%) and then radical deoxygenation gave **150** in 82% yield. The final product **22** was obtained by hydrogenation (81%).



OBn
HO
BnO
O
O(CH2)7CH3
OBn
86
1. NaH
2. CS2
3. CH3I
S
CH3S
O
OBn
BnO
O(CH2)7CH3
OBn
149
Bu3SnH
AIBN
OH
HO
O(CH2)7CH3
OH
22
H2, Pd-C
OBn
BnO
O(CH2)7CH3
OBn
150

Fig 62. Preparation of the 4-deoxy monosaccharide **22**.

Treatment of dibenzoate **69**, the fucosyl acceptor in the synthesis of the 6-deoxy disaccharide, with sodium methoxide gave the 6-deoxy monosaccharide **23** in 96% yield (Fig 63).



69 **23**

Fig 63. Preparation of the 6-deoxy monsaccharide **23**.

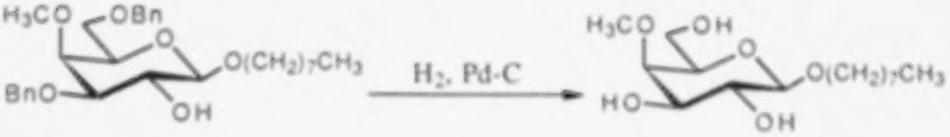
3. 2. 2 PREPARATION OF O-METHYL ANALOGS

The 4-O-methyl and 6-O-methyl derivatives, respectively **28** and **29**, were obtained by hydrogenation of the corresponding di-benzyl ethers used a fucosyl acceptors in the disaccharide syntheses. Thus **104** afforded **28** in 86% yield (Fig 64), and **29** was obtained from **107** by quantitative debenzylation (Fig 65).

3. 2. 3 PREPARATION OF AMINO ANALOGS

The amino derivative **32** was synthesized in 73% yield from the fully deprotected azide **126** by hydrogenation (Fig 66).

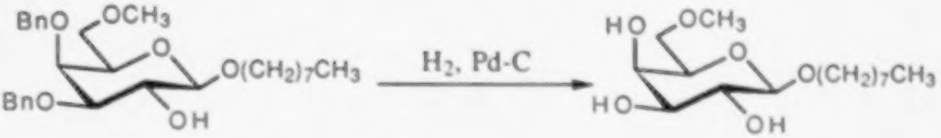
The preparation of the 6-amino derivative **34** began with alcohol **136**. Treatment with hydrazine acetate to remove the phthalimido group followed by hydrogenolysis of the benzyl ethers gave the product in 70% yield (Fig 67).


H3CO
OBn
O
O(CH2)7CH3
BnO
OH
H2, Pd-C
H3CO
OH
O
O(CH2)7CH3
HO
OH

104

28

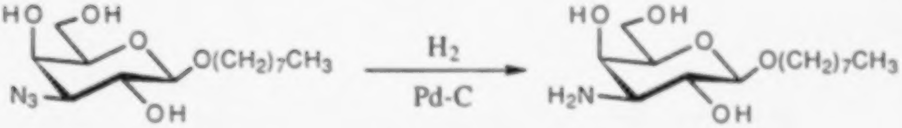
Fig 64. Preparation of the 4-O-methyl monsaccharide **28**.


BnO
OCH3
O
O(CH2)7CH3
BnO
OH
H2, Pd-C
HO
OCH3
O
O(CH2)7CH3
HO
OH

107

29

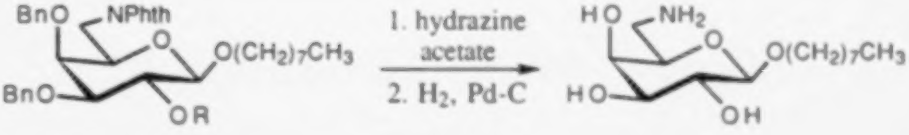
Fig 65. Preparation of the 6-O-methyl monsaccharide **29**.


HO
OH
O
O(CH2)7CH3
N3
OH
H2
Pd-C
HO
OH
O
O(CH2)7CH3
H2N
OH

126

32

Fig 66. Preparation of the 3-amino monosaccharide **32**.


BnO
NPhth
O
O(CH2)7CH3
BnO
OR
1. hydrazine acetate
2. H2, Pd-C
HO
NH2
O
O(CH2)7CH3
HO
OH

136

34

Fig 67. Preparation of the 6-amino monsaccharide **34**.

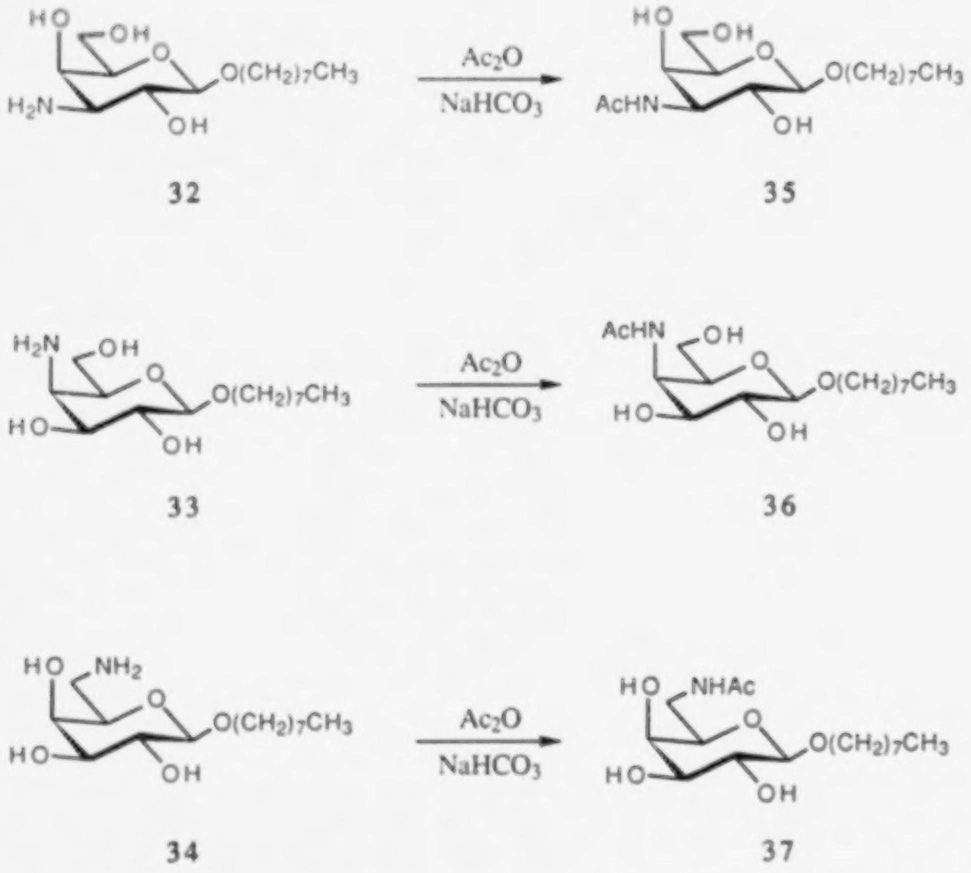

32
35
33
36
34
37
Ac2O
NaHCO3
O(CH2)7CH3
H2N
AcHN
NHAc
NH2

Fig 68. Preparation of acetamido monsaccharides **35-37**.

3. 2. 4 PREPARATION OF ACETAMIDO ANALOGS

The acetamido derivatives were prepared in the same manner as the corresponding disaccharide derivatives. Acylation of **32**, **33** or **34** with acetic anhydride and sodium bicarbonate in methanol, provided the acetamido derivatives **35**, **36**, **37** in quantitative yield by TLC (Fig 68).

3.3 ENZYMATIC TESTING OF MONOSACCHARIDE ANALOGS

3.3.1 POTENTIAL SUBSTRATE ABILITY OF MONOSACCHARIDES

The H-transferase used in these studies was a cloned α (1→2) fucosyltransferase obtained from Glycomed Inc. The enzyme was immobilized when received and the enzyme was therefore cleaved form the beads before use. The experimental portion of this thesis contains details about the cleavage protocol and cloning procedure used. In order to assure that the enzyme was similar to the natural protein, before screening these compounds with the enzyme, a K_m was determined for the natural substrate, octyl-β-D-galactopyranoside **1**. The value obtained, 6.4 ± 1.2 mM, is roughly twice that of the K_m of phenyl-β-D-galactopyranoside with a purified H-transferase from human serum[114]. This close agreement in K_m suggests that the cloned enzyme is at least similar to the natural protein. The V_{max} for **1** was determined to be 3.03 ± 0.2 pmol/min under the standard conditions described in the experimental section.

The results of the initial evaluation of monosaccharides **21-38** are shown in Table 9. In contrast to the A and B-transferases, the enzyme appears to recognize, at least to some extent, compounds with modifications at all three positions. Those analogs with modifications at C-3 show the least activity (0-2.5%). In all cases for a series of analogs, the C-4 modified analog is more active than the C-3 modified analog. As well, with the exception of the amino series the C-6 modified analogs are more active than the C-4 derivatives. In is interesting to note that three of the analogs, 4-O-methyl (**28**), 6-O-methyl (**29**) and 6-acetamido (**37**) derivatives are actually better substrates than the parent substrate **1**. The 6-fluoro analog (**25**) also retains most of the activity of **1**, and the 6-deoxy compound (**23**) is also a reasonable substrate. This latter observation confirms

Table 9. Relative acceptor activity of monosaccharides **1** and **21-38** towards the blood group H (Fuc)-transferase

Substrate	% Activity
Native **(1)**	100
3-Deoxy **(21)**	0.3
4-Deoxy **(22)**	1.6
6-Deoxy **(23)**	64
3-Fluoro **(24)**	0.5
4-Fluoro **(25)**	4.0
6-Fluoro **(26)**	93
3-O-Methyl **(27)**	2.1
4-O-Methyl **(28)**	123
6-O-Methyl **(29)**	316
3-Epimer **(30)**	0
4-Epimer **(31)**	0.5
3-Amino **(32)**	2.5
4-Amino **(33)**	8.6
6-Amino **(34)**	6.7
3-NHAc **(35)**	1.5
4-NHAc **(36)**	3.4
6-NHAc **(37)**	125
Ara **(38)**	27

[a] Compounds **1** and **21-38** were present at a concentration of 6.4 mM. Experiments were performed in duplicate with variation in replicates of 10% or less.

previously reported results[138]. The sterically less demanding arabino derivative (**38**) is a modest substrate. Only the 6-amino analog (**34**) is a poor substrate.

These results suggest that in the active site the area occupied by OH-4 and OH-6 is relatively nonpolar, and that the replacement of the polar OH with a nonpolar methyl group enhances recognition by the enzyme. A hydrophobic region in this area would also explain the relatively low activity of the **34** which shows an activity of only 6.7%. The positioning of a charge from the protonated amine in a hydrophobic area is likely to be disfavored resulting in the analog being a poor substrate. Also, the observation that the 6-acetamido derivative is recognized suggests that the enzyme will tolerate substitutions of large steric bulk at C-6.

The binding of hydrophilic molecules such as carbohydrates by hydrophobic areas of proteins has been studied previously[182, 183]. In order to explain this effect, Lemieux has suggested that the binding of these polar groups by hydrophobic surfaces is achieved by the formation of intramolecular hydrogen bonds. Of particular interest is that the studies performed have concentrated on galactose derivatives. For the case described here, it is possible that hydrogen bonding between the OH-6 and the ring oxygen and OH-4 and O-6 enable the polar molecule to be recognized by the hydrophobic portion of the active site (Fig 63). Methylation of either OH-4 or OH-6 would destroy one of these hydrogen bonds; however, if a hydrophobic pocket is indeed present in this enzyme, the energy lost appears to be more than compensated for by a favorable interaction with the hydrophobic methyl groups.

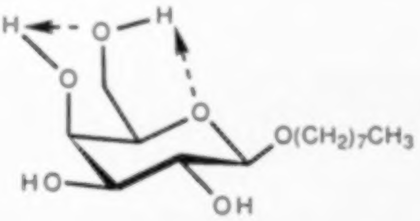


Fig 69. Intramolecular hydrogen bonding possible between Gal OH-4, Gal OH-6 and the ring oxygen.

3. 3. 2 POTENTIAL INHIBITOR ABILITY OF MONOSACCHARIDES

Those analogs not showing significant activity as substrates were tested for potential inhibitory activity. The results are presented in Table 10 where both percent inhibition and estimated K_i values are shown. Only two of the Gal OH-4 modified derivatives (**22** and **36**) have any inhibitory activity. The inhibition for these compounds is small and the estimated K_i's are an order of magnitude larger than the K_m of **1**. All of the Gal OH-3 modified derivatives posses inhibitory activity. The greatest inhibition is seen with the 3-epimeric analog (**30**) having an estimated K_i of 900 μM. Another reasonable inhibitor is the 3-amino derivative (**32**) which has an estimated K_i of 2.5 mM.

In the introduction, it was postulated that the H-enzyme, which transfers the carbohydrate with inversion of configuration, might have a base on the enzyme to help deprotonate the acceptor hydroxyl group thus facilitating S_N2 nucleophilic displacement. Similar to the 3-amino disaccharide described in Chapter 2, the ability of the amino compound to inhibit might be suggestive of a base on the enzyme near this part of the active site.

That the 3-epimer is a potent inhibitor can be rationalized by considering a previous investigation[72] with another inverting glycosyltransferase. In that report the enzyme was inhibited by a structural analog possessing an O-methyl substituent at the hydroxyl group adjacent to site of reactivity. It was proposed that the O-methyl group was inhibiting the enzyme by sterically blocking the sugar nucleotide from entering the active site. A similar mechanism may be in play here. If the sugar nucleotide binds from the bottom side of the galactose residue, an axially disposed hydroxyl group at C-3 might hinder the approach of the donor.

Table 10. Relative inhibitor activity and estimated K_i's of monosaccharides **21**-**38** towards the blood group H (Fuc)-transferase

Substrate	% Inhibition[a]	Estimated K_i[b] (mM)
3-Deoxy **(21)**	20	12.8
4-Deoxy **(22)**	8	36.8
3-Fluoro **(24)**	36	5.7
4-Fluoro **(25)**	0	NA
3-O-Methyl **(27)**	20	12.8
3-Epimer **(30)**	78	0.9
4-Epimer **(31)**	0	NA
3-Amino **(32)**	56	2.5
4-Amino **(33)**	0	NA
6-Amino **(34)**	0	NA
3-NHAc **(35)**	35	5.9
4-NHAc **(36)**	7	42.5

[a] Concentration of potential inhibitor was 6.4 µM with acceptor 1 at 6.4 µM. Experiments were performed in duplicate with variation in replicates of 10% or less.

[b] Estimated K_i assuming the inhibition is competitive and calculated from the equation $i = [I]/([I] + K_i\{1 + [S]/K_m\})$, where i is the fractional inhibition, $[I]$ the inhibitor concentration, and $[S]$ the substrate concentration.[175]

NA = not applicable

3. 3. 3 SUMMARY

These results indicate that, in general, Gal OH-3 and OH-4 are required for the compound to have a reasonable activity as a substrate. However, the 4-O-methyl compound is an exception to this generalization, which could possibly be explained by the favorable interaction of this hydrophobic group with a lipophilic region in the active site. Additionally, the 6-OH group can be replaced with little adverse effect provided that the substitution does not introduce a charge into the C-6 position.

The Gal OH-4 modified derivatives posses little activity either as acceptors or as inhibitors. This would suggest that, as in the A and B-transferases, this hydroxyl group is important in the recognition by the enzyme. However, the fact that some analogs of this structure can serve as either substrate or inhibitors indicates that the presence of this hydroxyl group is not absolutely critical.

All analogs modified at Gal OH-3 are inhibitors of the enzyme. The ability of the enzyme to recognize a wide range of substrates at this position indicates that even this close to the site of reactivity the enzyme is tolerant of functional group modifications.

3. 4. SUGGESTIONS FOR FUTURE WORK

Having now probed the active site of this enzyme with these monosaccharide derivatives, it is clear that in order for any compound to be a useful inhibitor of this enzyme the modifications should be done at the disaccharide level on Type I-VI disaccharides. The greater affinity of the enzyme for these substrates will provide a better chance of producing a tight binding inhibitor. Detailed enzyme kinetics on some of these more active monosaccharide analogs might provide further insight into the ways in which the enzyme recognizes the compounds. The detergent properties of these octyl glycosides, and the high concentrations required could possibly lead to artifacts, however.

More specific suggestions include probing further the proposed hydrophobic area around Gal OH-6. Replacement with even larger hydrophobic groups such as benzyl groups might lead to even greater activity. Modifications at C-2 could also be further explored. It has already been shown that a Type I disaccharide with Gal OH-2 deoxygenated produces a compound that serves as an inhibitor of a related enzyme from pig submaxillary gland[71]. The K_i of this deoxy compound is four times higher then the K_m of the native Type I structure. A disaccharide with an amino group at C-2 could also be prepared fairly easily from commercially available galactosamine and might lead to a potent inhibitor if there is indeed a base involved in the catalytic mechanism.

CHAPTER FOUR:

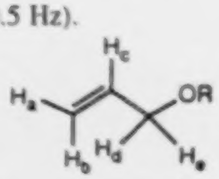
EXPERIMENTAL

4.1 CHEMICAL SYNTHESIS

4.1.1 GENERAL METHODS

Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22±2° C. Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with sulfuric acid. All commercial reagents were used as supplied and chromatography solvents were distilled prior to use. Column chromatography was performed on Silica Gel 60 (E. Merck 40-60 μM, Darmstadt). Millex-GV (0.22 μM) filter units were from Millipore (Missisuaga, ON), C_{18} Sep-Pak sample preparation cartridges were from Waters Associates (Missisuaga, ON) and Ecolite scintillation cocktail was from ICN Radiochemicals (St. Laurent PQ). UDP-[6-^{3}H]-Gal (specific activity 15 Ci/mmol) and UDP-[6-^{3}H]-GalNAc (specific activity 10 Ci/mmol) were from American Radiolabelled Chemicals (St. .Louis, MO). GDP-[1-^{3}H]-Fuc (specific activity 6.7 Ci/mmol) was from New England Nuclear (Wilmington, DE) UDP-Gal, UDP-GalNAc and ATP were from Sigma (St. Louis, MO). GDP-Fuc was prepared as previously described[181]. ^{1}H NMR were recorded at 360 MHz (Bruker WM-360) or 300 MHz (Bruker AM-300) with either internal $(CH_3)_4Si$ (δ 0, $CDCl_3$, CD_3OD) or DOH (δ 4.80, D_2O). ^{13}C NMR were recorded either at 75.5 MHz (Bruker AM-300) or 125.7 MHz (Varian Unity 500) with internal $(CH_3)_4Si$ (δ 0, $CDCl_3$, CD_3OD) or external 1,4 dioxane (δ 67.4, D_2O). ^{19}F NMR were recorded at 188.3 MHz (Bruker WH-200) or 376.5 MHz (Bruker WH-400) with external $CFCl_3$ (δ 0, $CDCl_3$, CD_3OD, D_2O). IR spectra were recorded on a Nicolet MX-1 instrument in $CHCl_3$. ^{1}H data are reported as though they were first order. All ^{13}C shifts assignments are tentative and were assigned based on comparison with published spectra[184-187]. Unless otherwise stated, all reactions were carried out at room temperature and in the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. Organic solutions were dried (sodium sulfate) prior to concentration under vacuum at < 40° C (bath). Microanalyses were carried out by the analytical services at this department and all samples submitted for elemental analyses were dried overnight under vacuum with phosphorus pentoxide at 56° C (refluxing acetone). Fast atom bombardment mass spectra were recorded on samples suspended in a matrix of either glycerol and hydrogen chloride or Cleland's matrix using a Kratos AEIMS9 instrument with xenon as the bombarding gas.

Protons of the allyl group present in the compounds described in the thesis were designated H_a, H_b, H_c, H_d and H_e as defined below. These protons showed the same coupling constants and thus the same multiplicity pattern in all the compounds examined, only the chemical shifts varied. The observed couplings were as follows: H_a, (dddd, $J_{a,c}$ 10.5 Hz, $J_{a,d} = J_{a,e} = J_{a,b}$ 1.5 ± 0.5 Hz). H_b, (dddd, $J_{b,c}$ 17.0 Hz, $J_{b,d} = J_{b,e} = J_{a,b}$ 1.5 ± 0.5 Hz). H_c, (dddd, $J_{b,c}$ 17.0 Hz, $J_{a,c}$ 10.5 Hz, $J_{c,d} = J_{c,e}$ 5.5 Hz). H_d, (dddd, $J_{d,e}$ 13.5 Hz, $J_{c,d}$ 5.5 Hz, $J_{b,d} = J_{a,d}$ 1.5 ± 0.5 Hz). H_e, (dddd, $J_{d,e}$ 13.5 Hz, $J_{c,e}$ 5.5 Hz, $J_{a,e} = J_{b,e}$ 1.5 ± 0.5 Hz).



4.1.2 SYNTHESIS OF DISACCHARIDE ANALOGS

Octyl 3-O-benzyl-β-D-galactopyranoside **(40)**. A solution of octyl β-D-galactopyranoside[158] (**39**, 10.02 g, 34.32 mmol) and dibutyl tin oxide (8.51 g, 34.20 mmol) was refluxed in benzene (250 mL) for 21 hours. Water was removed by passing the refluxing solvent through a column of 4 Å molecular sieves. To this solution was added tetra-N-butylammonium iodide (12.87 g, 34.88 mmol) and benzyl bromide (8.2 mL, 69 mmol) and refluxing continued for an additional 21 hours. The solution was evaporated to give a brown oil which was dissolved in dichloromethane and extracted with a saturated solution of sodium thiosulfate. After evaporation, column chromatography of the resulting oil (1:1 dichloromethane:ethyl acetate) gave **40** (7.8 g, 60%) as a white solid, $[\alpha]_D$ -3.1° (*c* 1.4, $CHCl_3$), R_f 0.46 (1:1 dichloromethane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.45 (m, 5 H, Ph), 4.74 (s, 2 H, $PhC\underline{H}_2$), 4.23 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.74-4.04 (m, 5 H, H-2, H-4, H-6a, H-6b, $OC\underline{H}_2CH_2$), 3.39-3.56 (m, 3 H, $OC\underline{H}_2CH_2$, H-5, H-3), 2.77 (d, 1 H, $J_{4,4\text{-}OH}$ 1 Hz, 4-OH), 2.56 (d, 1 H, $J_{2,2\text{-}OH}$ 2.5 Hz, 2-OH), 2.44 (dd, 1 H, $J_{6a,6\text{-}OH}$ 8.5 Hz, $J_{6b,6\text{-}OH}$ 4 Hz), 1.62 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 137.72 (Ph quaternary), 128.65, 128.13, 127.94 (Ph methine), 103.12 (C-1), 80.16 (C-3), 74.35 (C-5), 72.23 ($Ph\underline{C}H_2$), 71.17 (C-2), 70.19 ($O\underline{C}H_2CH_2$), 67.22 (C-4), 62.50 (C-6), 31.85, 29.65, 29.41, 29.25, 25.99, 22.67 (octyl CH_2), and 14.11 (octyl CH_3).

Anal. Calcd for $C_{21}H_{34}O_6$ (382.50): C, 65.97; H, 8.90. Found: C, 65.69; H, 8.98.

Octyl 3-O-benzyl-4,6-benzylidene-β-D-galactopyranoside (**41**). Galactoside **40** (6.56 g, 17.2 mmol) was dissolved in benzaldehyde (50 mL) and zinc chloride (3.79 g, 27.8 mmol) added. After stirring for five hours the solution was cooled to 0° C and water (125 mL) was added. Stirring continued for one hour and then the mixture was diluted with dichloromethane and extracted with sodium bicarbonate, water and brine. The organic layer was dried and evaporated under reduced pressure to remove the benzaldehyde. Column chromatography of the resulting clear oil (3:1 hexane:ethyl acetate) gave **41** (6.9 g, 86%) as a white solid, $[\alpha]_D$ + 32.8° (*c* 1.4, $CHCl_3$), R_f 0.14 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.60 (m, 10 H, Ph), 5.46 (s, 1 H, $PhC\underline{H}O_2$), 4.76 (s, 2 H, $PhC\underline{H}_2$), 4.30 (dd, 1 H, $J_{6a,6b}$ 12.5 Hz, $J_{5,6a}$ 1.5 Hz, H-6a), 4.28 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.12 (dd, 1 H, $J_{3,4}$ 3 Hz, $J_{4,5}$ 1 Hz, H-4), 4.02 (dd, 1 H, $J_{6a,6b}$ 12.5 Hz, $J_{5,6b}$ 1.5 Hz, H-6b), 3.94 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.44-3.54 (m, 2 H, H-3, $OC\underline{H}_2CH_2$), 3.34 (br. s, 1 H, H-5), 2.43 (br. s, 1 H, 2-OH), 1.55-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88

(t, 3 H J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.24, 137.66 (Ph quaternary), 128.89, 128.45, 128.10, 127.65 (Ph methine), 102.99 (C-1), 101.15 ($Ph\underline{C}HO_2$), 79.21 (C-3), 73.33 (C-4), 71.54 ($Ph\underline{C}H_2$), 70.14 (C-2), 69.85 ($O\underline{C}H_2CH_2$), 69.34 (C-6), 66.73 (C-5), 31.83, 29.55, 29.42, 29.24, 25.99, 22.67 (octyl CH_2), and 14.11 (octyl CH_3).

Anal. Calcd for $C_{28}H_{38}O_6$ (470.61): C, 71.46; H, 8.14. Found: C, 71.17; H, 8.00.

Octyl 3-O-benzyl-4,6-benzylidene-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside **(43)**. Alcohol **41** (50 mg, 0.11 mmol) and tetraethylammonium bromide (25 mg, 0.12 mmol) were dissolved in dichloromethane (4 mL) and dimethylformamide (0.5 mL) containing crushed 4 Å molecular sieves (3 g). The system was purged with argon allowed to stir overnight. To this slurry was added freshly prepared 2,3,4-tri-O-α-L-benzyl-fucopyranosyl bromide[174] (**42**, 0.44 mmol) in dichloromethane (3 mL) and the reaction allowed to stir for 2 days under argon. Methanol (1 mL) was added and stirring continued for thirty minutes and then the reaction was filtered and taken to dryness. Chromatography of the residue gave the product **43** (63 mg, 67%) as a white solid, $[\alpha]_D$ - 50.5° (*c* 0.6, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.17-7.60 (m, 25 H, Ph), 5.64 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 5.40 (s, 1 H, $PhC\underline{H}O_2$), 4.95, 4.86, 4.70, 4.65, 4.58, 4.54, 4.52 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.45 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.44 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 4.29 (dd, 1 H, $J_{6a,6b}$ 12 Hz, $J_{5,6a}$ 1 Hz, H-6a), 4.18 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 9.5 Hz, H-2),4.14 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 3.86-4.06 (m, 4 H, $OC\underline{H}_2CH_2$, H-2', H-3', H-6b), 3.81 (dd, 1 H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 3.64 (br d, 1 H, $J_{3'4'}$ 1.5 Hz, H-4'), 3.39 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.32 (br. s, 1 H, H-5'), 1.45-1.60 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.35 (10 H, octyl CH_2), 1.13 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 139.08, 138.88, 138.54, 138.35, 137.78 (Ph quaternary), 128.75, 128.27, 128.17, 128.09, 127.77, 127.46, 127.32, 127.29, 127.08, 126.28 (Ph methine), 101.82 (C-1), 100.87 ($Ph\underline{C}HO_2$), 97.01 (C-1'), 81.71(C-3), 79.55 (C-2), 78.20 (C-4'), 75.99 (C-4), 74.72, 73.05 ($Ph\underline{C}H_2$), 72.80 (C-3'), 72.47 ($Ph\underline{C}H_2$), 71.57 (C-2'), 70.59 ($O\underline{C}H_2CH_2$), 69.42 (C-5'), 69.35 (C-5), 66.39 ($Ph\underline{C}H_2$), 66.18 (C-6),31.86, 29.69, 29.33, 26.29, 22.65 (octyl CH_2), 16.59 (C-6'), and 14.10 (octyl CH_3).

Anal. Calcd for $C_{55}H_{66}O_{10}$ (887.13): C, 74.47; H, 7.50. Found: C, 74.45; H, 7.60.

Octyl 2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(2)**. The protected disaccharide **43** (105 mg, 0.12 mmol) was dissolved in methanol (10 mL), 5% palladium on carbon (50 mg) added and the solution stirred under a flow of hydrogen overnight. After completion of the reaction, the catalyst was filtered away and the solvent evaporated. The product was purified by redissolution in water and then passing the solution through a Waters C_{18} Sep-Pak cartridge. The cartridge was washed with water and then the product eluted with methanol. The methanol eluant was evaporated, the residue redissolved in water, filtered through a 0.22 µM filter and lyophilized to give the product **2** (47 mg, 90%) as a white solid.^{1}H NMR (CD_3OD): δ 5.10 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1'), 4.21 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.20 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.82 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.75 (d, 1 H, $J_{3,4}$ 3 Hz, H-4), 3.50-3.73 (m, 7 H, H-2, H-3, H-6a, H-6b, H-2', H-3', H-4'), 3.31-3.50 (m, 2 H, H-5, $OC\underline{H}_2CH_2$), 1.52-1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.22-1.43 (10 H, octyl CH_2), 1.18 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 103.56 (C-1), 101.57 (C-1'), 79.07 (C-2), 76.48 (C-5), 75.74 (C-3), 73.74 (C-3'), 72.56 (C-4'), 71.76 (C-3), 70.78 ($O\underline{C}H_2CH_2$), 70.62 (C-2'), 70.38 (C-4), 67.78 (C-5'), 62.38 (C-6), 32.98, 30.98, 30.60, 30.39, 27.29, 23.68 (octyl CH_2), 16.78 (C-6'), and 14.41 (octyl CH_3). FABMS (Glycerol-HCl): $m/z = 461$ $[M+Na]^+$ and 439 $[M+H]^+$ ($C_{20}H_{38}O_{10}$ requires $m/z = 438$).

Octyl 3-O-allyl-β-D-galactopyranoside **(44)**. A solution of octyl β-D-galactopyranoside[158] (**39**, 6.54 g, 22.39 mmol) and dibutyl tin oxide (5.53 g, 22.21 mmol) were refluxed in benzene (250 mL) for 24 hours as described for **40**. The solution was cooled to 60° C and then tetra-N-butylammonium iodide (8.28 g, 22.43 mmol) and allyl bromide (20 mL, 229 mmol) were added and heating at 60° C continued for an additional 20 hours. The solution was cooled, washed with a saturated solution of sodium thiosulfate and evaporated. After evaporation, column chromatography of the resulting oil (1:1 dichloromethane:ethyl acetate) gave **44** (4.51 g, 60%) as a white solid, $[\alpha]_D$ -5.1° (*c* 0.6, $CHCl_3$), R_f 0.26 (1:1 dichloromethane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 5.96 (1 H, H_c allyl), 5.33 (1 H, H_b allyl), 5.23 (1 H, H_a allyl), 4.26 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.21 (m, 2 H, H_d allyl, H_e allyl), 4.04 (ddd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 2 Hz, $J_{4,4\text{-OH}}$ 1 Hz, H-4), 3.78-4.01 (m, 3 H, $OC\underline{H}_2CH_2$, H-6a, H-6b), 3.73 (ddd, 1 H, $J_{4,5}$ 2 Hz, $J_{5,6a}$ 10 Hz, $J_{5,6b}$ 9 Hz, H-5), 3.46-3.58 (2 H, H-2, $OC\underline{H}_2CH_2$), 3.39 (dd, 1 H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 2.46 (d, 1 H, $J_{4,4\text{-OH}}$ 1 Hz, 4-OH), 2.25 (d, 1 H, $J_{2,2\text{-OH}}$ 2.5 Hz, 2-OH), 2.05 (dd, 1 H, $J_{6a,6\text{-OH}}$ 8.5 Hz, $J_{6b,6\text{-OH}}$ 4 Hz), 1.57-1.72 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 134.79 ($CH_2{=}\underline{C}HCH_2O$), 118.02 ($\underline{C}H_2{=}CHCH_2O$), 103.12 (C-1), 80.04 (C-3), 74.34 (C-5),71.08 ($CH_2{=}CH\underline{C}H_2O$), 70.91 (C-2), 70.20 ($O\underline{C}H_2CH_2$), 66.98 (C-4), 62.44 (C-6), 31.83, 29.63, 29.40, 29.24, 25.97, 22.66 (octyl CH_2), and 14.10 (octyl CH_3).

Anal. Calcd for $C_{17}H_{32}O_6$ (332.44): C, 61.42; H, 9.70. Found: C, 61.33; H, 9.45.

Octyl 3-O-allyl-2,4,6-tri-O-benzyl-β-D-galactopyranoside **(45)**. Allyl ether **44** (4.41 g, 13.29 mmol) was dissolved in dry dimethylformamide (100 mL). Sodium hydride (4.06 g, 80% dispersion in oil, 135.4 mmol) was added and the mixture stirred for 30 minutes. Benzyl bromide (15 mL, 120 mmol) was added and stirring continued for 15 hours. The solution was then cooled to 0° C, water added, and then diluted with dichloromethane and extracted with sodium bicarbonate, water and brine. Evaporation of the organic layer gave a brown liquid which was chromatographed (12:1 hexane:ethyl acetate) to give **45** (5.73 g, 71%) as a colorless oil, $[\alpha]_D$ -3.0° (*c* 1.1, $CHCl_3$), R_f 0.25 (12:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.2-7.4 (m, 15 H, Ph), 5.93 (1 H, H_c allyl), 5.32 (1 H, H_b allyl), 5.17 (1 H, H_a allyl), 4.93, 4.89, 4.74, 4.61, 4.45, 4.39 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.32 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.19 (m, 2 H, H_d allyl, H_e allyl), 3.91 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.85 (dd, 1 H, $J_{3,4}$ 3 Hz, $J_{4,5}$ 1 Hz, H-4), 3.74 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10 Hz, H-2),3.44-3.62 (m, 4 H, H-5, H-6a, H-6b, $OC\underline{H}_2CH_2$), 3.40 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3 Hz, H-3), 1.55-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} =7 Hz, octyl CH_3). ^{13}C NMR: δ 138.93, 138.75, 138.01 (Ph quaternary), 135.06 ($CH_2{=}\underline{C}HCH_2O$), 128.56, 128.38, 128.32, 128.20, 128.10, 127.10, 127.86, 127.72, 127.46 (Ph methine), 116.50 ($\underline{C}H_2{=}CHCH_2O$), 103.96 (C-1), 81.99 (C-3), 79.54 (C-2), 75.13, 74.39, 73.54 (Ph $\underline{C}H_2$), 73.47 (C-5), 73.38 (C-4), 71.92 ($CH_2{=}CH\underline{C}H_2O$), 70.02 ($O\underline{C}H_2CH_2$), 68.96 (C-6), 31.82, 29.74, 29.43, 29.25, 26.16, 22.65 (octyl CH_2), and 14.09 (octyl CH_3).

Anal. Calcd for $C_{38}H_{50}O_6$ (602.82): C, 75.72; H, 8.36. Found: C, 75.46; H, 8.33.

Octyl 2,4,6-tri-O-benzyl-β-D-galactopyranoside **(46)**. To a solution of **45** (5.73 g, 9.520 mmol) dissolved in 7:3:1 ethanol:benzene:water (125 mL), tris(triphenylphosphine)rhodium (I) chloride (1.26 g, 1.36 mmol) and 1,4-diazabicyclo[2.2.2]octane (460 mg, 4.10 mmol) were added and the solution refluxed for 20 hours. The solvent was evaporated and the residue dissolved in 9:1 acetone:water (50 mL). Mercuric oxide (50 mg) and mercuric chloride (12 g) were added and stirring continued overnight. The reaction was then diluted with dichloromethane and washed with saturated potassium iodide, water and brine. Evaporation of the organic layer followed by chromatography (6:1 hexane:ethyl acetate)

gave **46** (4.54 g, 85 %) as a colorless oil, $[\alpha]_D$ + 5.7° (*c* 1, $CHCl_3$), R_f 0.20 (6:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.2-7.4 (m, 15 H, Ph), 4.97, 4.78, 4.67, 4.62, 4.50, 4.43 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.33 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.93 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.85 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 3.59-3.70 (m, 4 H, H-3, H-5, H-6a, H-6b), 3.55 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10 Hz, H-2), 3.48 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.40 (d, 1 H, $J_{3,3\text{-}OH}$ 4.5 Hz, 3-OH), 1.58-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.59, 138.02 (Ph quaternary), 128.51, 128.46, 128.34, 128.22, 128.18, 127.83, 127.70 (Ph methine), 103.84 (C-1), 79.68 (C-2), 75.62 (C-5), 75.02, 74.66 ($Ph\underline{C}H_2$), 74.16 (C-4), 73.64 (C-3), 73.58 ($Ph\underline{C}H_2$), 70.07 ($O\underline{C}H_2CH_2$), 68.87 (C-6), 31.87, 29.77, 29.46, 29.30, 26.22, 22.70 (octyl CH_2), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{35}H_{46}O_6$ (562.75): C, 74.70; H, 8.24. Found: C, 74.55; H, 7.93.

Octyl 2,4,6-tri-O-benzyl-3-O-[(methylthio)thiocarbonyl]-β-D-galactopyranoside **(47)**. To a solution of **46** (1.01 g, 1.80 mmol) in dry tetrahydrofuran (15 mL) was added sodium hydride (162 mg, 80% in oil, 5.4 mmol) and imidazole (32 mg). After stirring for one hour, carbon disulfide (1.1 mL, 18.1 mmol) was added. One hour later, methyl iodide (340 µL, 5.5 mmol) was added and stirring continued overnight. Evaporation of the solvent gave a yellow liquid which was chromatographed (6:1 hexane:ethyl acetate) to give **47** (1.0 g, 86%) as a yellow oil, $[\alpha]_D$ + 51.9° (*c* 0.9, $CHCl_3$), R_f 0.55 (12:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.15-7.45 (m, 15 H, Ph), 5.75 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3 Hz, H-3), 4.85, 4.69, 4.65 (d,1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.36-4.5 (m, 3 H, 3 $PhC\underline{H}_2$, H-1), 4.18 (br d, 1 H, $J_{3,4}$ 3 Hz, H-4), 3.89-3.99 (m, 2 H, H-2, $OC\underline{H}_2CH_2$), 3.68 (br t, 1 H, $J_{5,6a} = J_{5,6b}$ 6.5 Hz, H-5), 3.45-3.63 (m, 3 H, H-6a, H-6b, $OC\underline{H}_2CH_2$), 2.54 (s, 3 H, SCH_3) 1.58-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).

^{13}C NMR ($CDCl_3$): δ 215.66 (C=S), 138.38, 137.97, 137.91 (Ph quaternary), 128.46, 128.37, 128.29, 128.27, 128.09, 127.86, 127.80, 127.75, 127.60 (Ph methine), 103.76 (C-1), 84.00 (C-3), 76.90 (C-2), 74.87, 74.79, 73.57 ($PhC\underline{H}_2$), 73.36 (C-5), 72.90 (C-4), 70.28 ($O\underline{C}H_2CH_2$), 68.52 (C-6), 31.86, 29.73, 29.45, 29.30, 26.17, 22.70 (octyl CH_2), 19.16 (SCH_3), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{37}H_{48}O_6S_2$ (652.91): C, 68.07; H, 7.41; S, 9.82. Found: C, 68.06; H, 7.20; S, 9.76.

Octyl 2,4,6-tri-O-benzyl-3-deoxy-β-D-xylo-hexopyranoside **(48)**. Compound **47** (717 mg, 1.10 mmol) was dissolved in dry toluene (100 mL) and then tributylstannane (1.5 mL, 5.58 mmol) and AIBN (135 mg, 0.82 mmol) were added. The solution was heated under reflux for 60 minutes. Evaporation of the solvent followed by chromatography (12:1 hexane:ethyl acetate) gave **48** (403 mg, 67%) as a colorless oil, $[\alpha]_D$ - 26.1° (*c* 0.4, $CHCl_3$), R_f 0.32 (12:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.2-7.4 (m, 15 H, Ph), 4.86, 4.61, 4.56, 4.55, 4.46 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.38 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.36 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 3.95 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.54-3.72 (m, 5 H, H-2, H-4, H-5, H-6a, H-6b), 3.49 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.36 (ddd, 1 H, $J_{3a,3e}$ 14.5 Hz, $J_{2,3e}$ 5 Hz, $J_{3e,4}$ 3 Hz, H-3e), 1.58-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.46, (ddd, 1 H, $J_{3a,3e}$ 14.5 Hz, $J_{2,3a}$ 2.4 Hz, $J_{3a,4}$ 11.5 Hz, H-3a), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.91, 138.33, 138.25 (Ph quaternary), 128.37, 128.30, 128.26, 127.90, 127.87, 127.72, 127.63, 127.58, 127.50 (Ph methine), 105.46 (C-1), 76.48 (C-2), 73.55, 73.21 ($Ph\underline{C}H_2$), 73.10 (C-5), 72.47 (C-4), 71.11 ($Ph\underline{C}H_2$), 69.45 ($O\underline{C}H_2CH_2$), 69.34 (C-6), 32.83 (C-3), 31.85, 29.78, 29.45, 29.29, 26.21, 22.68 (octyl CH_2), and 14.11 (octyl CH_3).

Anal. Calcd for $C_{35}H_{46}O_5$ (546.75): C, 76.89; H, 8.48. Found: C, 76.96; H, 8.81.

Octyl 3-deoxy-β-D-xylo-hexopyranoside **(21)**. Compound **48** (512 mg, 0.94 mmol), was dissolved in methanol (15 mL) and 5% palladium on carbon (250 mg) added. The reaction was allowed to stir overnight under a flow of hydrogen. The catalyst was filtered away, the solvent evaporated and the residue chromatographed (19:1 dichloromethane:methanol) to give **21** (237 mg, 92%) as a white solid $[\alpha]_D$ -55.9° (c 0.6, CH_3OH), R_f 0.30 (19:1 dichloromethane:methanol). ^1H NMR ($CDCl_3$): δ 4.25 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.03 (m, 1 H, H-4), 3.93 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.75-3.90 (m, 3 H, H-2, H-6a, H-6b), 3.46-3.58 (m 2 H, H-5, $OC\underline{H}_2CH_2$), 2.29 (ddd, 1 H, J_{gem} 14.5 Hz, $J_{2,3e}$ 5.5 Hz, $J_{3e,4}$ 3.5 Hz, H-3e), 1.55-1.77 (m, 3 H, H-3a, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 105.75 (C-1), 77.11 (C-2), 70.05 ($O\underline{C}H_2CH_2$), 67.88 (C-5), 66.02 (C-4), 63.34 (C-6), 37.14 (C-3), 31.83, 29.68, 29.42, 29.26, 26.01, 22.67 (octyl CH_2), and 14.10 (octyl CH_3).

Anal. Calcd for $C_{14}H_{28}O_5$ (276.38): C, 60.84; H,10.21. Found: C, 60.74; H, 10.42

Octyl 4,6-benzylidene-3-deoxy-β-D-xylo-hexopyranoside **(49)**. Galactoside **21** (100 mg, 0.37 mmol) was dissolved in benzaldehyde (1 mL) and dichloromethane (2 mL) and zinc chloride (75 mg, 0.55 mmol) added. After stirring for three hours the solution was cooled to 0° C and water (2 mL) was added. Stirring continued for one hour and then the mixture was diluted with dichloromethane and extracted with 2M NaOH (to remove traces of benzoic acid), water and brine. The organic layer was dried with sodium sulfate and evaporated under reduced pressure to remove the benzaldehyde. Column chromatography of the resulting clear oil (3:1 hexane:ethyl acetate) gave **49** (112 mg, 83%) as a white solid, $[\alpha]_D$ - 80.4° (c 0.5, $CHCl_3$), R_f 0.70 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.50-7.62 (m, 2 H, Ph), 7.34-7.44 (m, 3 H, Ph), 5.56 (s, 1 H, $PhC\underline{H}O_2$), 4.34 (dd, 1 H, $J_{6a,6b}$ 12.5 Hz, $J_{5,6a}$ 1 Hz, H-6a), 4.28 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.10 (m, 2 H, H-4, H-6b), 4.02 (dt, 1 H, J_{gem} 10

Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.92 (ddd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3e}$ 5 Hz, $J_{2,3a}$ 10.5 Hz, H-2), 3.51 (m, 2 H, H-5, $OC\underline{H}_2CH_2$), 2.42 (ddd, 1 H, $J_{3a,3e}$ 14 Hz, $J_{2,3e}$ 5 Hz, $J_{3e,4}$ 2.5 Hz, H-3e), 2.30 (br. s, 1 H, 2-OH), 1.73 (ddd, 1 H, $J_{3a,3e}$ 14 Hz, $J_{2,3a}$ 10.5 Hz, $J_{3a,4}$ 3.5 Hz, H-3a), 1.58-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR: δ 138.06 (Ph quaternary), 129.03, 128.23, 126.40 (Ph methine), 105.27 (C-1), 101.37 ($Ph\underline{C}HO_2$), 73.20 (C-4), 69.70 ($O\underline{C}H_2CH_2$), 69.55 (C-6), 69.35 (C-2), 65.81 (C-5), 35.25 (C-3), 31.87, 29.63, 29.47, 29.28, 26.08, 22.70 (octyl CH_2), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{21}H_{33}O_5$ (365.49): C, 69.20; H, 8.85. Found: C, 69.51; H, 8.85.

Octyl 4,6-benzylidene-3-deoxy-2-O- (2,3,4-tri-O-benzyl-α-D-fucopyranosyl)-β-D-xylo-hexopyranoside **(50)**. Alcohol **49** (130 mg, 0.35 mmol) and tetraethylammonium bromide (81 mg, 0.39 mmol) were fucosylated as described for **41** using 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.408 mmol). Column chromatography of the mixture (3:1 hexane:ethyl acetate) gave the disaccharide **50** (241 mg, 88%) as a white solid ($[\alpha]_D$ - 111.51° (*c* 0.5, $CHCl_3$), R_f 0.34 (3:1 hexane:ethyl acetate).^1H NMR ($CDCl_3$): δ 7.10-7.55 (m, 20 H Ph), 5.48 (s, 1 H, $PhC\underline{H}O_2$), 4.98 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.90 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.60-4.88 (m 5 H, $PhC\underline{H}_2$), 4.47 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.20-4.35 (m, 2 H, H-5', H-6a), 3.88-4.08 (m, 6 H, H-2', H-3', H-6b, H-4, H-2, $OC\underline{H}_2CH_2$), 3.65, 1 H, $J_{3'4'}$ 2 Hz, H-4'), 3.32-3.46 (m, 2 H, H-5, $OC\underline{H}_2CH_2$), 2.38 (ddd, 1 H, J_{gem} 14 Hz, $J_{2,3e}$ 5.5 Hz, $J_{3e,4}$ 2.5 Hz, H-3e), 1.75 (ddd, 1 H, $J_{3a,4}$ 3.5 Hz, $J_{2,3a}$ 11 Hz, J_{gem} 13 Hz, H-3a), 1.55-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.45 (10 H, octyl CH_2), 1.11 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.98, 138.78, 138.58, 137.69 (Ph quaternary), 129.16, 128.91, 128.60, 128.29, 128.19, 128.10, 127.93, 127.71, 127.62, 127.42, 127.38, 127.33, 126.37 (Ph methine), 103.18 (C-1), 101.25 ($Ph\underline{C}HO_2$), 94.27 (C-1'), 79.67 (C-4'), 78.03 (C-3'), 76.38 (C-3), 75.91

(C-2'), 74.72, 73.37, 73.16 (Ph$\underline{C}$H$_2$), 73.03 (C-4),69.48 (O$\underline{C}$H$_2$CH$_2$), 68.99 (C-6), 68.83 (C-5'), 68.25 (C-5), 33.41 (C-3), 31.83, 29.72, 29.49, 29.33, 26.22, 22.62 (octyl CH$_2$), 16.50 (C-6'), and 14.08 (octyl CH$_3$).

Anal. Calcd for $C_{48}H_{60}O_9$ (781.01): C, 73.82; H, 7.74. Found: C, 73.67; H, 7.74.

Octyl 3-deoxy-2-O-(α-L-fucopyranosyl)-β-D-xylo-hexopyranoside **(3)**. The protected disaccharide **50** (150 mg, 0.19 mmol) was dissolved in methanol (20 mL), 5% palladium on carbon (75 mg) added and the solution stirred under a flow of hydrogen overnight. The catalyst was filtered away, and the product purified as described for **2** to give **3** (70 mg, 86%) as a white solid. ^{1}H NMR (CD$_3$OD): δ 4.84 (br. s, 1 H, H-1'), 4.40 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.33 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.41-4.00 (m, 10 H, H-2, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4', 2 OC$\underline{H}_2$CH$_2$), 2.29 (dt, 1 H, $J_{3e,3a}$ 13 Hz, $J_{3e,4}$ $J_{3e,2}$ 4 Hz, H-3e), 1.50-1.70 (m, 3 H, H-3a, OCH$_2$C$\underline{H}_2$), 1.23-1.45 (10 H, octyl CH$_2$), 1.18 (d, 3 H, $J_{5'6'}$ = 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH$_3$). ^{13}C NMR (CD$_3$OD): δ 104.90 (C-1), 96.81 (C-1'), 79.48 (C-2), 73.79 (C-5), 71.73 (C-3'), 70.31 (C-4'), 70.13 (O$\underline{C}$H$_2$CH$_2$), 69.83 (C-2'), 67.34 (C-4), 66.88 (C-5), 62.81 (C-6), 35.93 (C-3), 33.02, 31.03, 30.61, 30.48, 27.44, 23.70 (octyl CH$_2$), 16.63 (C-6'), and 14.42 (octyl CH$_3$). FABMS (Glycerol-HCl): *m/z* = 445 [M+Na]$^+$ and 423 [M+H]$^+$ ($C_{20}H_{38}O_9$ requires *m/z* = 422).

Octyl 2-O-allyl-4,6-benzylidene-3-O-benzyl-β-D-galactopyranoside **(51)**. To a solution of **41** (4.77 g, 10.15 mmol) in dry dimethylformamide (75 mL), sodium hydride (730 mg, 30.45 mmol) was added. After stirring for thirty minutes allyl bromide (3.5 mL, 40.6 mmol) was added and stirring continued for 15 hours. The mixture was diluted with dichloromethane and extracted with water, sodium bicarbonate and brine. Column chromatography of the resulting oil (3:1 hexane:ethyl acetate) gave **51** (4.68 g, 90%) as a white solid, $[\alpha]_D + 29.7^o$ (*c* 1.3, CHCl$_3$), R$_f$ 0.44 (3:1 hexane:ethyl acetate). ^{1}H NMR (CDCl$_3$): δ 7.20-7.60 (m, 10 H, Ph), 5.98 (1 H, H$_c$

allyl), 5.48 (s, 1 H, $\mathrm{PhC\underline{H}O_2}$), 5.30 (1 H, $\mathrm{H_b}$ allyl), 5.15 (1 H, $\mathrm{H_a}$ allyl), 4.80, 4.73 (d, 1 H, J_{gem} 11.5 Hz, $\mathrm{PhC\underline{H}_2}$), 4.41 (1 H, $\mathrm{H_d}$ allyl), 4.27 (m, 2 H, H-6a, $\mathrm{H_e}$ allyl), 4.29 (d, 1 H, $J_{1,2}$, 8.0 Hz, H-1), 4.07 (dd, 1 H, $J_{3,4}$ 3.8 Hz, $J_{4,5}$ 1 Hz, H-4), 3.99 (dd, 1 H, $J_{6a,6b}$ 12.5 Hz, $J_{5,6b}$ 1.5 Hz, H-6b), 3.95 (dt, 1H, J_{gem} 10 Hz, J_{vic} 7 Hz, $\mathrm{OC\underline{H}_2CH_2}$), 3.69 (dd, 1 H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10 Hz, H-2), 3.48 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.8 Hz, H-3), 3.45 (dt, 1 H, J_{gem} 10 Hz, J_{vic} = 7 Hz, $\mathrm{OC\underline{H}_2CH_2}$), 3.28 (br. s, 1 H, H-5), 1.62 (m, 2 H, $\mathrm{OCH_2C\underline{H}_2}$), 1.20-1.40 (10 H, octyl $\mathrm{CH_2}$), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl $\mathrm{CH_3}$). ^{13}C NMR ($\mathrm{CDCl_3}$): δ 138.66, 137.97 (Ph quaternary), 135.46 ($\mathrm{CH_2{=}\underline{C}HCH_2O}$), 128.66, 128.33, 128.08, 127.61, 126.58 (Ph methine), 116.41 ($\mathrm{\underline{C}H_2{=}CHCH_2O}$), 103.75 (C-1), 101.33 ($\mathrm{Ph\underline{C}HO_2}$), 79.13 (C-3), 78.11 (C-2), 74.25 (C-4), 73.96 ($\mathrm{CH_2{=}CH\underline{C}H_2O}$), 72.12 ($\mathrm{Ph\underline{C}H_2}$), 70.03 ($\mathrm{O\underline{C}H_2CH_2}$), 69.29 (C-6), 66.42 (C-5), 31.85, 29.68, 29.43, 29.28, 26.10, 22.68 (octyl $\mathrm{CH_2}$), and 14.11 (octyl $\mathrm{CH_3}$).

Anal. Calcd for $\mathrm{C_{31}H_{42}O_6}$ (510.68): C, 72.91; H, 8.29. Found: C, 72.95; H, 8.18.

Octyl 2-O-allyl-3,6-di-O-benzyl-β-D-galactopyranoside (**52**). Compound **51** (3.10 g, 6.09 mmol), sodium cyanoborohydride (4.15 g, 66.1 mmol) and methyl orange indicator were dissolved in dry tetrahydrofuran (40 mL) containing crushed 3 Å molecular sieves (2 g). The solution was cooled to 0° C and then ethereal hydrogen chloride was added until the red color of the solution persisted. After four hours, TLC indicated the reaction was complete and the reaction was quenched by the addition of sodium bicarbonate. The reaction was filtered, diluted with dichloromethane and washed with water, brine and evaporated. Column chromatography of the residue (3:1 hexane:ethyl acetate) gave **52** (2.54 g, 81%) as a colorless oil, $[\alpha]_D$ - 8.5° (*c* 0.8, $\mathrm{CHCl_3}$), R_f 0.50 (3:1 hexane:ethyl acetate). ^{1}H NMR ($\mathrm{CDCl_3}$): δ 7.20-7.40 (m, 10 H, Ph), 5.96 (1 H, $\mathrm{H_c}$ allyl), 5.28 (1 H, $\mathrm{H_b}$ allyl), 5.16 (1 H, $\mathrm{H_a}$ allyl), 4.75, 4.71 (d, 1 H, J_{gem} = 11.5 Hz, $\mathrm{PhC\underline{H}_2}$), 4.58 (s, 2 H, $\mathrm{PhC\underline{H}_2}$), 4.39 (1 H, $\mathrm{H_d}$ allyl), 4.27 (d, 1

H, $J_{1,2}$ 7.8 Hz, H-1), 4.21 (m, 1 H, H_e allyl), 3.99 (ddd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, $J_{4,4\text{-}OH}$ 2 Hz, H-4), 3.91 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.79 (dd, 1 H, $J_{6a,6b}$ 10 Hz $J_{5,6a}$ 6 Hz, H-6a), 3.70 (dd, 1 H, $J_{6a,6b}$ 10 Hz $J_{5,6b}$ 6 Hz, H-6b), 3.45-3.57 (3 H, H-2, H-5, $OC\underline{H}_2CH_2$), 3.42 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3), 2.45 (d, 1 H, $J_{4,4\text{-}OH}$ 2 Hz, 4-OH), 1.55-1.65 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.12 (Ph quaternary), 135.28 ($CH_2{=}\underline{C}HCH_2O$), 128.47, 128.44, 127.82, 127.78, 127.75 (Ph methine), 116.73 ($\underline{C}H_2{=}CHCH_2O$), 103.71 (C-1), 80.51 (C-3), 78.68 (C-2), 73.94 ($CH_2{=}CH\underline{C}H_2O$), 73.73 ($Ph\underline{C}H_2$), 73.20 (C-5), 72.56 ($PhC\underline{H}_2$), 70.02 ($O\underline{C}H_2CH_2$), 69.28 (C-6), 67.11 (C-4), 31.84, 29.72, 29.40, 29.27, 26.10, 22.68 (octyl CH_2), and 14.11 (octyl CH_3).

Anal. Calcd for $C_{31}H_{44}O_6$ (512.69): C, 72.62; H, 8.65. Found: C, 72.29; H, 8.75.

Octyl 2-O-allyl-3,6-di-O-benzyl-4-O-[(methylthio)thiocarbonyl]-β-D-galactopyranoside **(53)**. To a solution of **52** (353 mg, 0.69 mmol) in 20 mL dry tetrahydrofuran was added sodium hydride (68 mg, 80% in oil, 2.27 mmol) and imidazole (5 mg). After stirring for one hour, carbon disulfide (420 µL, 6.96 mmol) was added and stirring continued for another hour. At this point methyl iodide (129 µL, 2.07 mmol) was added and stirring continued overnight. Evaporation of the solvent gave a yellow liquid which was chromatographed (4:1 hexane:ethyl acetate) to give **53** (332 mg, 80%) as a yellow oil, $[\alpha]_D$ + 23.5° (*c* 0.6, $CHCl_3$), R_f 0.55 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.40 (m, 10 H, Ph), 6.48 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, H-4), 5.93 (1 H, H_c allyl), 5.26 (1 H, H_b allyl), 5.13 (1 H, H_a allyl), 4.78, 4.53, 4.50, 4.45 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.35 (1 H, H_d allyl), 4.35 (1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.22 (1 H, H_e allyl), 3.92 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.76 (t, 1 H, $J_{5,6a}$ $J_{5,6b}$ 6 Hz, $J_{4,5}$ 1 Hz H-5), 3.43-3.63 (5 H, H-2, H-3, H-6a, H-6b, $OC\underline{H}_2CH_2$), 2.58 (s, 3 H, $SC\underline{H}_3$), 1.62 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl

CH_2), and 0.88 (t, 3 H J_{vic} 7 Hz, octyl $C\underline{H}_3$). ^{13}C NMR ($CDCl_3$): δ 216.61 (C=S), 137.97, 137.83 (Ph quaternary), 135.22 ($CH_2=\underline{C}HCH_2O$), 128.43, 128.26, 128.03, 127.95, 127.81, 127.63 (Ph methine), 116.70 ($\underline{C}H_2=CHCH_2O$), 103.87 (C-1), 79.41 (C-3), 78.65 (C-2), 76.47 (C-4), 74.15 ($CH_2=CH\underline{C}H_2O$), 73.85 ($Ph\underline{C}H_2$), 72.89 (C-5), 72.73 ($PhC\underline{H}_2$), 70.51 ($O\underline{C}H_2CH_2$), 68.69 (C-6), 67.11 (C-5), 31.86, 29.69, 29.40, 29.28, 26.06, 22.69 (octyl CH_2), 19.23 (SCH_3), and 14.12 (octyl CH_3).

Anal. Calcd for $C_{33}H_{46}O_6S_2$ (602.85): C, 65.75; H, 7.69; S, 10.64. Found: C, 65.69; H, 7.57; S, 10.35.

Octyl 2-O-allyl-3,6-di-O-benzyl-4-deoxy-β-D-xylo-hexopyranoside **(54)**. Compound **53** (157 mg, 0.26 mmol) was dissolved in dry toluene (50 mL) and then tributylstannane (1.05 mL, 3.9 mmol) and AIBN (35 mg, 0.21mmol) were added. The solution was heated under reflux for 90 minutes. Evaporation of the solvent followed by chromatography (99:1 dichloromethane:methanol) gave **54** (109 mg, 85%) as a colorless oil, $[\alpha]_D$ - 19.9° (*c* 0.5, $CHCl_3$), R_f 0.45 (99:1 dichloromethane:methanol). ^1H NMR ($CDCl_3$): δ 7.20-7.40 (m, 10 H, Ph), 5.98 (1 H, H_c allyl), 5.29 (1 H, H_b allyl), 5.15 (1 H, H_a allyl), 4.72, 4.66, 4.59, 4.52 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.40 (1 H, H_d allyl), 4.27 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.23 (1 H, H_e allyl), 3.91 (dt, 1 H, J_{gem} 10 Hz, J_{vic} = 7 Hz, $OC\underline{H}_2CH_2$), 3.43-3.63 (5 H, H-3, H-5, H-6a, H-6b, $OC\underline{H}_2CH_2$), 3.18 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, H-2), 2.08 (ddd, 1 H, $J_{4e,5}$ 1 Hz, $J_{3,4e}$ 5 Hz, J_{gem} 13 Hz, H-4e), 1.57-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.50 (11 H, octyl CH_2, H-4a), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): 138.74, 138.15 (Ph quaternary), 135.37 ($CH_2=\underline{C}HCH_2O$), 128.36, 128.30, 127.64, 127.56, 127.48 (Ph methine), 116.57 ($\underline{C}H_2=CHCH_2O$), 103.78 (C-1), 82.59 (C-3), 78.09 (C-2), 73.73 ($CH_2=CH\underline{C}H_2O$), 73.50, 72.55 ($PhC\underline{H}_2$), 72.37 (C-6), 70. 93 (C-5), 70.06 ($O\underline{C}H_2CH_2$), 34.04 (C-4), 31.80, 29.73, 29.36, 29.23, 26.06, 22.63 (octyl CH_2), and 14.08 (octyl CH_3).

Anal. Calcd for $C_{31}H_{44}O_5$ (496.69): C, 74.96; H, 8.93. Found: C, 74.68; H, 8.83.

Octyl 3,6-di-O-benzyl-4-deoxy-β-D-xylo-hexopyranoside **(55)**. To a solution of **54** (199 mg, 0.40 mmol) dissolved in 7:3:1 ethanol:benzene:water (20 mL), tris(triphenylphosphine)rhodium (I) chloride (55 mg, 0.06 mmol) and 1,4 diazabicyclo[2.2.2]octane (20 mg, 0.18 mmol) were added and the solution refluxed for 20 hours. The solvent was evaporated and the residue dissolved in 9:1 acetone water (10 mL). Mercuric oxide (3 mg) and mercuric chloride (1 g) were added and stirring continued at room temperature overnight. The reaction was then diluted with dichloromethane and washed with saturated potassium iodide, water and brine. Evaporation of the organic layer followed by chromatography (4:1 hexane:ethyl acetate) gave **55** (159 mg, 86%) as a colorless oil, $[\alpha]_D$ - 8.2° (*c* 0.6, $CHCl_3$), R_f 0.45 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.40 (m, 10 H, Ph), 4.72, 4.65, 4.53 (d, 1 H, J_{gem} 11.5 Hz, PhC$\underline{H}_2$), 4.21 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.89 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OC$\underline{H}_2$CH$_2$), 3.40-3.68 (6 H, H-2, H-3, H-5, H-6a, H-6b, OC$\underline{H}_2$CH$_2$), 2.46 (d, 1 H, $J_{2,2\text{-}OH}$ 1.5 Hz, 2-OH), 2.12 (ddd, 1 H, $J_{4e,5}$ 1.8 Hz, $J_{3,4e}$ 4.5 Hz, J_{gem} 13 Hz, H-4e), 1.55-1.68 (m, 2 H, OCH$_2$C$\underline{H}_2$), 1.43 (dt, 1 H, $J_{3,4a}$ $J_{4a,5}$ 11 Hz, J_{gem} 13 Hz, H-4a), 1.20-1.40 (10 H, octyl CH$_2$), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH$_3$). ^{13}C NMR ($CDCl_3$): 138.46 (Ph quaternary), 128.46, 128.43, 127.71 (Ph methine), 103.00 (C-1), 77.94 (C-3), 75.10 (C-5), 73.57, 72.49 (PhC$\underline{H}_2$), 71.68 (C-6), 71.31 (C-2), 70.08 (O$\underline{C}$H$_2$CH$_2$), 33.31 (C-4), 31.83, 29.67, 29.40, 29.24, 26.00, 22.66 (octyl CH$_2$), and 14.11 (octyl CH$_3$).

Anal. Calcd for $C_{28}H_{40}O_5$ (456.63): C, 73.65; H, 8.83. Found: C, 73.35; H, 8.28.

Octyl 4-deoxy-2-O-(α-L-fucopyranosyl)-β-D-xylo-hexopyranoside **(4)**. Alcohol **55** (101 mg, 0.22 mmol) and tetraethylammonium bromide (51 mg, 0.243 mmol) were dissolved in dichloromethane (4 mL) and dimethylformamide (0.5 mL) containing crushed 4 Å molecular sieves (3 g) and the solution stirred overnight. To this slurry was added freshly prepared 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.105 mmol) in dichloromethane (3 mL) and the reaction allowed to stir for 2 days. Methanol (1 mL) was added and stirring continued for thirty minutes and then the reaction was filtered and taken to dryness. At this point it was not possible to obtain a pure product, therefore the partially purified product **156** was dissolved in methanol (10 mL), 5% palladium on carbon (50 mg) added and the solution stirred under a flow of hydrogen overnight. Final purification as described for **2** gave **4** (36 mg, 39%) as a white solid. ^{1}H NMR (CD_3OD): δ 5.19 (d, 1 H, $J_{1'2'}$ 2 Hz, H-1'), 4.30 (d, 1 H $J_{1,2}$ 8 Hz, H-1), 4.29 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.88 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.72-3.84 (m, 3 H, H-2', H-3, H-6a), 3.63 (d, 1 H, $J_{3'4'}$ 1.5 Hz, H-3'), 3.44-3.58 (m, 4 H, H-5, H-2, H-4', $OC\underline{H}_2CH_2$), 3.22 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, H-2), 1.90 (ddd, 1 H, $J_{4e,5}$ 1.5 Hz, $J_{3,4e}$ 5 Hz, $J_{4e,4a}$ 13 Hz, H-4e), 1.62 (m, 2 H, $OCH_2C\underline{H}_2$), 1.25-1.45 (11 H, octyl CH_2, H-4a), 1.19 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 103.36 (C-1), 101.77 (C-1'), 83.42 (C-2), 73.79 (C-4'), 73.76 (C-5), 73.02 (C-3), 71.75 (C-3'), 70.83 ($O\underline{C}H_2CH_2$), 70.68 (C-2'), 67.83 (C-5'), 65.50 (C-6), 36.49 (C-4), 33.01, 30.99, 30.63, 30.41, 27.30, 23.70 (octyl CH_2), 16.80 (C-6'), and 14.42 (octyl CH_3). FABMS (Glycerol-HCl): *m/z* = 445 $[M+Na]^+$ and 423 $[M+H]^+$ ($C_{20}H_{38}O_9$ requires *m/z* = 422).

Methyl 3,4 di-O-benzoyl-6-bromo-6-deoxy-β-D-galactopyranoside **(64)**. Methyl 4,6-benzylidene-3-O-benzoyl-β-D-galactopyranoside (**63**, 3.0 g, 7.78 mmol), N-bromosuccinimide (1.73 g, 9.72 mmol) and barium carbonate (7 g) were refluxed in carbon tetrachloride (75 mL) for 30 minutes. The orange solution was cooled, filtered, evaporated and the resulting liquid chromatographed (1:1 hexane:ethyl acetate) to give **64** (2.74 g, 76%) as a white foam, $[\alpha]_D$ + 71.6° (*c* 0.4, $CHCl_3$), R_f 0.65 (1:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.0-7.84 (m, 10 H, Ph), 5.74 (dd, 1 H, $J_{3,4}$

3.2 Hz, $J_{4,5}$ 1 Hz, H-4), 5.20 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.2 Hz, H-3), 4.31 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.96 (m, 2 H, H-2, H-5), 3.57 (s, 3 H, OCH_3), 3.37 (dd, 1 H, $J_{6a,6b}$ 9.5 Hz, $J_{5,6a}$ 7 Hz, H-6a), 3.32 (dd, $J_{6a,6b}$ 9.5 Hz, $J_{5,6b}$ 6.5 Hz, H-6), 2.48 (br s, 1 H, 2-OH), ^{13}C NMR ($CDCl_3$): δ 165.94, 164.44 (C=O), 133.61, 133.26, 129.97, 129.81 (Ph methine), 129.25, 129.07 (Ph quaternary),128.59, 128.28 (Ph methine),104.27 (C-1), 73.92 (C-5),73.38 (C-3), 69.73 (C-2), 68.75 (C-4), 57.62 (OCH_3), 28.61 (C-6).

Anal. Calcd for $C_{21}H_{21}BrO_7$ (465.30): C, 54.21; H, 4.55; Br, 17.17. Found: C, 54.05; H, 4.64; Br, 17.32.

Methyl 3,4 di-O-benzoyl-6-deoxy-β-D-galactopyranoside (**65**). Compound **64** (2.43 g, 5.23 mmol), triethylamine (910 μL, 6.53 mmol) and 5% palladium on carbon (2.25 g) were stirred in 5:1 ethanol:ethyl acetate (120 mL) under a flow of hydrogen for 18 hours. The catalyst was filtered off, the solvent evaporated and the residue chromatographed (1:1 hexane:ethyl acetate) to give **65** (1.84 g, 91%) as a white solid, $[\alpha]_D + 100.0^o$ (*c* 0.4, $CHCl_3$), R_f 0.58 (1:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.2-8.1 (m, 10 H, Ph), 5.63 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, H-4), 5.34 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.40 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.05 (ddd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, $J_{2,2\text{-}OH}$ 2.5 Hz, H-2), 4.01 (q, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 3.65 (s, 3 H, OCH_3), 2.50 (d, 1 H, $J_{2,2\text{-}OH}$ 2.5 Hz, 2-OH), 1.32 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 166.06, 165.96 (C=O), 133.37, 133.15, 129.25, 129.78 (Ph methine), 129.45 (Ph quaternary), 128.49, 128.25 (Ph methine), 104.24 (C-1), 73.75 (C-5), 71.21 (C-3), 69.80 (C-2), 68.68 (C-4), 57.50 (OCH_3), 16.28 (C-6).

Anal. Calcd for $C_{21}H_{22}O_7$ (386.41): C, 65.28; H, 5.74. Found: C, 65.28; H, 5.97.

Methyl 2-O-acetyl-3,4 di-O-benzoyl-6-deoxy-β-D-galactopyranoside (**66**). Alcohol **65** (1.77 g, 4.58 mmol), was dissolved in pyridine (15 mL) and then acetic anhydride (5 mL) added. The reaction was stirred overnight and was quenched by cooling to 0° C and adding methanol. The solvent was evaporated and the product chromatographed (4:1 hexane:ethyl acetate) to give the product **66** (1.88 g, 96%) as a white foam, $[\alpha]_D$ + 139.4° (c 0.8, $CHCl_3$), R_f 0.22 (4:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.2-8.1 (m, 10 H, Ph), 5.67 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, H-4), 5.49 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10.5 Hz, H-2), 5.36 (dd, 1 H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.54 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.02 (dq, 1 H, $J_{5,6}$ 6.5 Hz, $J_{4,5}$ 1 Hz, H-5), 3.60 (s, 3 H, OCH_3), 1.98 (s, 3 H, acetate CH_3), 1.33 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 169.67 (acetate C=O), 165.98, 165.68 (benzoate C=O), 133.40, 133.30, 129.99, 129.76 (Ph methine), 129.27, 129.02 (Ph quaternary), 128.57, 128.38 (Ph methine), 102.0 (C-1), 72.24 (C-5), 71.04 (C-3), 69.65 (C-2), 69.17 (C-4), 56.95 (OCH_3), 20.81 (acetate CH_3), 16.27 (C-6).

Anal. Calcd for $C_{23}H_{24}O_8$ (428.44): C, 64.48; H, 5.65. Found: C, 64.27; H, 5.67.

2-O-acetyl-3,4 di-O-benzoyl-6-deoxy-α-D-galactopyranosyl chloride **(67)**. Compound **66** (901 mg, 2.10 mmol), and zinc chloride (50 mg) were dissolved in chloroform (2 mL). To this solution was added dichloromethyl methyl ether (5 mL) and the reaction was refluxed for 45 minutes. The solvent was evaporated and the product subjected to a rapid chromatographic separation (4:1 hexane:ethyl acetate) to give **67** (703 mg, 78%) as a white foam, R_f 0.47 (4:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.30-8.12 (m, 10 H, Ph), 6.50 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.84 (dd, 1 H, $J_{1,2}$ 4 Hz, $J_{2,3}$ 10 Hz, H-2), 5.80 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, H-4), 5.60 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.68 (q, 1 H, $J_{5,6}$ 6.5 Hz, $J_{4,5}$ 1 Hz, H-5), 2.08 (s, 3 H, acetate CH_3), 1.32 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 170.33 (acetate C=O), 165.72, 165.39 (benzoate C=O), 133.60, 133.35, 129.90, 129.74 (Ph methine), 129.13 (Ph quaternary), 128.66, 128.40 (Ph methine),92.02 (C-1), 70.98 (C-5), 68.47 (C-3), 68.32 (2C, C-4, C-2), 20.72 (acetate CH_3), 15.87 (C-6).

Octyl 2-O-acetyl-3,4 di-O-benzoyl-6-deoxy-β-D-galactopyranoside **(68)**. Silver triflate (502 mg, 1.95 mmol, dried in vacuo over potassium pentoxide for 1 hour), was stirred with collidine (125 µL, 1.02 mmol) and n-octanol (604 µL, 3.84 mmol) in dichloromethane (10 mL) continuing crushed 3 Å molecular sieves (2.5 g) under nitrogen at -30° C for 20 minutes. To this solution was added dropwise, chloride **67** (549 mg, 1.28 mmol) in dichloromethane (15 mL). The reaction was stirred under nitrogen and warmed to room temperature. After eight hours the reaction was quenched with collidine (200 µL), filtered and evaporated. The residue was then chromatographed (4:1 hexane:ethyl acetate) to give the product **68** (590 mg, 88%) as an oil, $[\alpha]_D$ + 114.7° (*c* 0.5, $CHCl_3$), R_f 0.51 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.28-8.11 (m, 10 H, Ph), 5.65 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, H-4), 5.48 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, H-2), 5.36 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.59 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.92-4.05 (m, 2 H, $OC\underline{H}_2CH_2$, H-5), 3.54 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.98 (s, 3 H, acetate CH_3), 1.55-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 13 H, octyl CH_2, H-6), 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 169.54 (acetate C=O), 166.07, 165.73 (benzoate C=O), 133.39, 133.29, 130.06, 129.82 (Ph methine), 129.34, 129.11 (Ph quaternary), 128.53, 128.40 (Ph methine), 101.30 (C-1), 72.29 (C-5), 71.13 (C-3), 70.19 ($O\underline{C}H_2CH_2$), 69.65 (C-2), 69.36 (C-4), 31.85, 29.53, 29.36, 29.31, 25.91, 22.69 (octyl CH_2), 20.79 (acetate CH_3), 16.35 (C-6), and 14.12 (octyl CH_3).

Anal. Calcd for $C_{30}H_{38}O_8$ (526.63): C, 68.42; H, 7.27. Found: C, 68.58; H, 7.52.

Octyl 3,4 di-O-benzoyl-6-deoxy-β-D-galactopyranoside **(69)**. Compound **68** (399 mg, 0.76 mmol) was dissolved in 49:1 methanol:acetyl chloride (9 mL) and stirred for 9 hours. The reaction was quenched with bicarbonate and then diluted with dichloromethane and extracted with bicarbonate, water and brine. Chromatography of the residue (4:1 hexane:ethyl acetate) gave **69** (331 mg, 90%) as an oil, $[\alpha]_D$ + 81.4° (*c*

0.4, $CHCl_3$), R_f 0.36 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-8.15 (m, 10 H, Ph), 5.65 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.38 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.49 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.07 (ddd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10 Hz, $J_{2,2\text{-}OH}$ 2.5 Hz), 4.02 (m, 2 H, $OC\underline{H}_2CH_2$, H-5), 3.62 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.38 (d, 1 H, $J_{2,2\text{-}OH}$ 2.5 Hz, 2-OH), 1.58-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 13 H, octyl CH_2, H-6), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 166.03 (C=O), 133.37, 133.13, 130.01, 129.82 (Ph methine), 129.57 (Ph quaternary), 128.51, 128.28 (Ph methine), 103.28 (C-1), 73.67 (C-5), 71.34 (C-3), 70.59 ($O\underline{C}H_2CH_2$), 69.88 (C-2), 69.72 (C-4), 31.85, 29.63, 29.43, 29.28, 25.99, 22.69 (octyl CH_2), 16.36 (C-6), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{28}H_{36}O_7$ (484.59): C, 69.40; H, 7.49. Found: C, 69.49; H, 7.64.

Octyl 3,4-di-O-benzoyl-6-deoxy-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**70**). Alcohol **69** (148 mg, 0.31 mmol) and tetraethylammonium bromide (71 mg, 0.34 mmol) were fucosylated as described for compound **41** using 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.224 mmol). Column chromatography of the mixture (3:1 hexane:ethyl acetate) gave the disaccharide **70** (245 mg, 88%) as an oil $[\alpha]_D$ - 1.3° (*c* 0.2, $CHCl_3$), R_f 0.24 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): 6.9-8.1 (m, 25 H Ph), 5.68 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, H-4), 5.57 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3), 5.40 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.98, 4.78 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.60-4.70 (m 3 H, H-1, 2 $PhC\underline{H}_2$), 4.44 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 4.28-4.38 (m, 3 H, 2 $PhC\underline{H}_2$, H-2, 3.94-4.04 (m, 2 H, H-5, $OC\underline{H}_2CH_2$), 3.86-3.94 (m, 2 H, H-3', H-2'), 3.68 (s, 1H, H-4'), 3.62 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.55-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.45 (13 H, octyl CH_2, H-6), 1.10 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 166.08, 165.30 (C=O), 138.68, 138.64, 137.82 (Ph quaternary), 133.24, 133.17, 129.92, 129.65 (Ph methine), 129.43, 129.31 (Ph quaternary), 128.42, 128.39 (Ph methine),102.04 (C-1), 97.08 (C-1'), 79.37 (C-4'), 77.99 (C-3'), 75.63 (C-2'), 75.50 (C-2), 74.72, 73.22, 72.36 ($Ph\underline{C}H_2$), 71.41 (C-3), 71.28 (C-4), 70.24 ($O\underline{C}H_2CH_2$), 69.22 (C-5), 66.55 (C-5'), 31.82, 29.66, 29.47, 29.30, 26.20, 22.63 (octyl CH_2), 16.55 (C-6), 16.27 (C-6'), and 14.08 (octyl CH_3).

Anal. Calcd for $C_{55}H_{64}O_{11}$ (901.12): C, 73.31; H, 7.16. Found: C, 73.05; H, 7.01.

Octyl 6-deoxy-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(5)**. To a solution of protected disaccharide **70** (200 mg, 0.22 mmol) in methanol (10 mL), 5% palladium on carbon (100 mg) was added and the solution stirred under a flow of hydrogen overnight. After completion of the reaction, the catalyst was filtered away and the solvent evaporated. The residue was redissolved in methanol (20 mL) and 1 M NaOH (1 mL) added and the reaction stirred overnight. The solution was neutralized with Amberlite IR 120 H (+) resin, evaporated and the product purified by chromatography (9:1 dichloromethane:methanol). Final purification was achieved as described for **2** to give the product **5** (76 mg, 81%) as a white solid, R_f 0.15 (9:1 dichloromethane:methanol). ^1H NMR (CD_3OD): δ 5.19 (d, 1 H, $J_{1'2'}$ 2 Hz, H-1'), 4.30 (d, 1 H $J_{1,2}$ 8 Hz, H-1), 4.29 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.83 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.56-3.77 (m, 7 H, H-2, H-3, H-4, H-5, H-2', H-3', H-4'), 3.48 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.53-1.66 (m, 2 H, $OCH_2C\underline{H}_2$), 1.21-1.42 (13 H, octyl CH_2, H-6), 1.18 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 103.38 (C-1), 101.54 (C-1'), 78.86 (C-2), 75.88 (C-5), 73.74 (C-3), 73.12 (C-4'), 71.76 (2 C, C-3' C-4'), 70.70 ($O\underline{C}H_2CH_2$), 70.61 (C-2'), 67.74 (C-5'), 32.99, 30.99, 30.59, 30.38, 27.28, 23.68 (octyl CH_2), 16.77 (C-6'), 16.67 (C-6), and 14.40 (octyl CH_3). FABMS (Glycerol-HCl): *m/z* = 445 $[M+Na]^+$ and 423 $[M+H]^+$ ($C_{20}H_{38}O_9$ requires *m/z* = 422).

2,4,6 tri-O-acetyl-3-deoxy-3-fluoro-α-D-galactopyranosyl bromide **(76)**. 1,2,4,6 tetra-O-acetyl-3-deoxy-3-fluoro-D-galactopyranose[164] **75** (0.86 g, 2.44 mmol), was dissolved in dichloromethane (2 mL) and hydrobromic acid (33% in acetic acid, 7 mL) added. The reaction was stirred for one hour and then evaporated to dryness, coevaporating with dry toluene. The crude product was purified by a rapid chromatographic separation (3:1 hexane:ethyl acetate) to give the product **76** (850 mg, 80%) as an oil which solidified on standing, R_f 0.48 (3:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 6.68 (t, 1 H, $J_{1,2} = J_{1,F}$ 4 Hz, H-1), 5.68 (ddd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, $J_{H4,F}$ 6 Hz, H-4), 5.11 (ddd, 1 H, $J_{1,2}$ 4.0 Hz, $J_{2,3}$ 10 Hz, $J_{H2,F}$ =12 Hz, H-2),5.00 (ddd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, $J_{H3,F}$ 48 Hz, H-3), 4.44 (br. t, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 4.24 (ddd, 1 H, $J_{5,6a}$ 6.5 Hz, $J_{6a,6b}$ 12 Hz, H-6a), 4.11 (ddd, 1 H, $J_{5,6b}$ 6.5 Hz, $J_{6a,6b}$ 12 Hz, $J_{H6b,F}$ 1 Hz, H-6b), 2.19 (s, 3 H, acetate CH_3), 2.08 (s, 6 H, acetate CH_3).

Octyl 2,4,6 tri-O-acetyl-3-deoxy-3-fluoro-β-D-galactopyranoside **(77)**. Compound **76** (644 mg, 1.74 mmol), was glycosylated as described for the conversion of **67** to **68**, using silver triflate (669 mg, 2.60 mmol), collidine (167 μL, 1.31 mmol) and octanol (830 μL, 5.22 mmol). The reaction was complete after 30 minutes. Chromatography of the crude reaction mix (4:1 hexane:ethyl acetate) gave the product **77** (448 mg, 62%) as an oil, $[\alpha]_D$ + 3.5° (*c* 1.2, $CHCl_3$), R_f 0.38 (4:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 5.55 (ddd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, $J_{4,F}$ 6 Hz, H-4), 5.28 (ddd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 9.5 Hz, $J_{2,F}$ 12 Hz, H-2), 4.61 (ddd, 1 H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.5 Hz, $J_{H3,F}$ 47 Hz, H-3), 4.40 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.17 (d, 2 H, $J_{5,6a}$ $J_{5,6b}$ 6.5 Hz, H-6a, H-6b), 3.88 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.83 (ddt, 1 H, $J_{5,6}$ 6.5 Hz, $J_{4,5}$ 1 Hz, $J_{5,F}$ 1 Hz), 3.47 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.18, 2.12, 2.07 (s, 3 H, acetate $C\underline{H}_3$), 1.45-1.70 (m, 2 H, $OC\underline{H}_2CH_2$), 1.20-1.40 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 170.43 170.02, 169.25 (acetate C=O), 100.77 (d, 1 C, $J_{C1,F}$ = 10.6 Hz, C-1), 88.98 (d, 1 C, $J_{C3,F}$ = 194.0 Hz, C-3), 70.35 ($O\underline{C}H_2CH_2$), 70.03 (d, 1 C, $J_{C2,F}$ = 25.7 Hz, C-2), 69.94 (C-5), 67.00 (d, 1 C, $J_{C4,F}$ = 16.6 Hz, C-4), 61.37 (d, 1 C, $J_{C6,F}$ = 2.4 Hz, C-6),31.82, 29.42, 29.29, 25.82, 22.66 (octyl CH_2), 20.79, 20.68, (acetate CH_3), and 14.09 (octyl CH_3). ^{19}F NMR ($CDCl_3$): δ -200.3 (ddd, 1 F, $J_{H3,F}$ 47 Hz, $J_{H2,F}$12 Hz, $J_{H4,F}$ 5.3 Hz, F-3).

Anal. Calcd for $C_{20}H_{36}FO_8$ (423.50): Calculated: C, 56.72; H, 8.57. Found: C, 57.30; H, 8.34.

Octyl 3-deoxy-3-fluoro-β-D-galactopyranoside **(24)**. Galactoside **77** (338 mg, 0.80 mmol), was dissolved in methanol (10 mL) and sodium methoxide (60 mg) added. After stirring for 48 hours, the solution was neutralized by the addition of prewashed Amberlite IR 120 (H+) resin. Evaporation of the solvent followed by chromatography (19:1 dichloromethane: methanol) gave the product **24** (218 mg, 92%) as a white solid, $[\alpha]_D$ -19.2° (*c* 0.7, CH_3OH), R_f 0.11 (19:1 dichloromethane: methanol). ^1H NMR (CD_3OD): δ 4.28 (ddd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{2,3}$ 9.5 Hz, $J_{H3,F}$ 48.5 Hz, H-3), 4.14 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.01 (dd, 1 H, $J_{H4,F}$ 6.5 Hz, $J_{3,4}$ 3.5 Hz, H-4), 3.86 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.62-3.80 (m, 2 H, H-2, H-6a, H-6b), 3.50 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.45 (br. t, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 1.58-1.75 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.31 (d, 1 C, $J_{C1,F}$ 11.8 Hz, C-1), 95.01 (d, 1 C, $J_{C3,F}$ 184.8 Hz, C-3), 75.25 (d, 1 C, $J_{C5,F}$ 6.9 Hz, C-5), 71.02 ($O\underline{C}H_2CH_2$), 70.98 (d, 1 C, $J_{C2,F}$ 18.1 Hz, C-2), 68.34 (d, 1 C, $J_{C4,F}$ 16.5 Hz, C-4), 62.03 (d, 1 C, $J_{C6,F}$ 3 Hz, C-4), 33.00, 30.80, 30.55, 30.41, 27.09, 23.71 (octyl CH_2), and 14.41 (octyl CH_3). ^{19}F NMR (CD_3OD): δ -201.0 (dddd, 1 F, $J_{H3,F}$ 48.5 Hz, $J_{H2,F}$ 13 Hz, $J_{H4,F}$ 6.5 Hz, $J_{H5,F}$ 1 Hz, F-6).

Anal. Calcd for $C_{14}H_{27}FO_5$ (294.37): C, 57.13; H, 9.25. Found: C, 56.88; H, 9.16.

Octyl 4,6-benzylidene-3-deoxy-3-fluoro-β-D-galactopyranoside **(78)**. Compound **24** (131 mg, 0.45 mmol) and benzaldehyde dimethyl acetal (201 μL, 1.34 mmol) were dissolved in acetonitrile (5 mL) and p-toluenesulfonic acid (5 mg) added. After stirring for 30 minutes the reaction was neutralized with triethylamine, evaporated and chromatographed (3:1 hexane:ethyl acetate), to give **78** (169 mg, 98%) as a white solid, $[\alpha]_D$ - 34.3° (*c* 0.5, $CHCl_3$), R_f 0.13 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.1-7.52 (m, 5 H, Ph), 5.42 (s, 1 H, $PhC\underline{H}O_2$), 4.4 (ddd, 1 H, $J_{2,3}$ 9 Hz, $J_{3,4}$ 3.5 Hz, $J_{H3,F}$ 45.5 Hz, H-3), 4.20-4.32 (m, 2 H, H-6a, H-4), 4.18 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.92-4.04 (m, 2 H, H-6b, H-2), 3.89 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.42 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.41 (br s., 1 H, H-5), 2.42 (d, 1 H, $J_{2,2\text{-}OH}$ 2-OH), 1.62 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 137.47 (Ph quaternary), 129.09, 128.18, 126.36 (Ph methine), 102.44 (d, 1 C, $J_{C1,F}$ 11.3 Hz, C-1), 101.04 ($Ph\underline{C}HO_2$), 91.21 (d, 1 C, $J_{C3,F}$ 191.7 Hz, C-3), 74.14 (d, 1 C, $J_{C4,F}$ 15.8 Hz, C-4), 70.31 ($O\underline{C}H_2CH_2$), 69.57 (d, 1 C, $J_{C2,F}$ 18.1 Hz, C-2), 69.01 (C-6), 65.62 (d, 1 C, $J_{C5,F}$ 6.0 Hz, C-5), 31.83, 29.52, 29.41, 29.24, 25.97, 22.67 (octyl CH_2), and 14.11 (octyl CH_3). ^{19}F NMR ($CDCl_3$): δ -205.1 (m, 1 F, F-3).

Anal. Calcd for $C_{21}H_{31}FO_5$ (382.48): C, 65.95; H, 8.17 Found: C, 65.84; H, 8.49.

Octyl 4,6-benzylidene-3-deoxy-3-fluoro-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside **(79)**. Alcohol **78** (117 mg, 0.31 mmol) and tetraethylammonium bromide (72 mg, 0.34 mmol) were fucosylated as described for compound **41** using 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.27 mmol). Column chromatography of the residue (3:1 hexane:ethyl acetate) gave the disaccharide **79** (217 mg, 89%) as an oil $[\alpha]_D$ - 84.0° (*c* 0.1, $CHCl_3$), R_f 0.23 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.2-7.6 (m, 20 H Ph), 5.55 (s, 1 H, $PhC\underline{H}O_2$), 5.36 (d, 1 H, $J_{1'2'}$ 4 Hz, H-1'), 4.99, 4.90 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.64-4.87 (m, 7

H, 6 $PhC\underline{H}_2$, H-3), 4.21-4.45 (m, 5 H, H-1, H-5', H-2, H-4, H-6a), 4.00-4.11 (m, 2 H, H-6b, H-2'), 3.87-3.98 (m, 2 H, H-3', $OC\underline{H}_2CH_2$), 3.66 (d, 1 H, $J_{3',4'}$ 2 Hz, H-4'), 3.40 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.36 (br s., 1 H, H-5), 1.45-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2), 1.12 (d, 1 H, $J_{5',6'}$ 6.5 Hz, H-6'), 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 139.15, 138.84, 138.47, 137.39 (Ph quaternary), 129.08, 128.34, 128.31, 128.27, 128.20, 128.18, 128.14, 127.59, 127.49, 127.42, 127.36, 126.42 (Ph methine), 101.06 ($PhC\underline{H}O_2$), 101.00 (d, 1 C, $J_{C1,F}$ 10 Hz, C-1), 97.10 (d, 1 C, $J_{C1',F}$ 5.3 Hz, C-1'), 93.36 (d, 1 C, $J_{C3,F}$ 191 Hz, C-3), 79.41 (C-4'), 78.10 (C-3'), 76.39 (C-2'), 74.78 ($Ph\underline{C}H_2$), 74.49 (d, 1 C, $J_{C4,F}$ 15.8 Hz, C-2), 73.38, 72.77 ($Ph\underline{C}H_2$), 72.11 (d, 1 C, $J_{C2,F}$ 16.5 Hz, C-4), 69.61 ($O\underline{C}H_2CH_2$), 69.06 (C-6), 66.45 (C-5'), 65.32 (d, 1 C, $J_{C5,F}$ 6.8 Hz, C-5), 31.87, 29.67, 29.51, 29.34, 26.21, 22.68 (octyl CH_2), 16.60 (C-6'), 14.13 (octyl CH_3),^{19}F NMR ($CDCl_3$): δ -203 (dt, 1 F, $J_{H3,F}$ 47 Hz, $J_{H4,F}$ 6 Hz, $J_{H2,F}$ 12.5 Hz, F-3).

Anal. Calcd for $C_{48}H_{59}FO_9$ (799.00): C, 72.16; H, 7.44. Found: C, 72.15; H, 7.59.

Octyl 3-deoxy-3-fluoro-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(6)**. The protected disaccharide **79** (166 mg, 0.21 mmol) was dissolved in methanol (10 mL) and 5% palladium on carbon (100 mg) was added and the solution stirred under a flow of hydrogen overnight. After completion of the reaction, the catalyst was filtered away, the solvent evaporated and the product was purified by chromatography (9:1 dichloromethane:methanol). Final purification of the product was achieved as described for **2** to give the product **6** (75 mg, 82%) as a white solid, R_f 0.20 (9:1 dichloromethane:methanol). ^1H NMR (CD_3OD): δ 5.12 (d, 1 H, $J_{1,2}$ 2 Hz, H-1'), 4.66 (ddd, 1 H, $J_{H3,F}$ 48.5 Hz, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.38 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.37 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 4.06 (dd, 1 H, $J_{H4,F}$ 7.5 Hz, $J_{3,4}$ 3.5 Hz, H-4), 3.98 (ddd, 1 H, $J_{H2,F}$ 7.5 Hz, $J_{1,2}$ 8 Hz, $J_{2,3}$ 9.5 Hz, H-2), 3.91 (dt, 1 H, J_{gem}

10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.68-3.79 (m, 4 H, H-6a, H-6b, H-2', H-3'), 3.63 (br. d, 1 H, $J_{3',4'}$ 1 Hz, H-4'), 3.44-3.55 (m, 2 H, H-5, $OC\underline{H}_2CH_2$), 1.52-1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.22-1.43 (10 H, octyl CH_2), 1.18 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 102.59 (d, 1 C, $J_{C1,F}$ 12.8 Hz, C-1), 99.83 (d, 1 C, $J_{C1',F}$ 6 Hz, C-1'), 96.60 (d, 1 C, $J_{C3,F}$ 184.0 Hz, C-3), 75.15 (d, 1 C, $J_{C5,F}$ 6.9 Hz, C-5), 74.01 (d, 1 C, $J_{C2,F}$ 16.6 Hz, C-2), 73.75 (C-3'), 71.53 (C-4'), 70.78 ($O\underline{C}H_2CH_2$), 69.97 (C-2'), 68.50 (d, 1 C, $J_{C4,F}$ 16.5 Hz, C-4), 67.44 (C-5'), 61.94 (d, 1 C, $J_{C6,F}$ 3.1 Hz, C-6), 32.99, 30.97, 30.57, 30.41, 27.38, 23.67 (octyl CH_2), 16.62 (C-6'), and 14.40 (octyl CH_3). ^{19}F NMR (CD_3OD): δ -198.4 (dt, 1 F, $J_{H3,F}$ 48.5 Hz, $J_{H2,F} = J_{H4,F}$ 7.5 Hz, F-3). FABMS (Glycerol:HCl): $m/z = 463$ $[M+Na]^+$ and 441 $[M+H]^+$ ($C_{20}H_{37}FO_9$ requires $m/z = 440$).

Octyl 4,6-benzylidene-2,3-di-O-benzyl-β-D-glucopyranoside (**85**). Octyl β-D-glucopyranoside (**31**, 1.05 g, 3.6 mmol) was dissolved in benzaldehyde (10 mL) and zinc chloride (750 mg, 5.4 mmol) added. After stirring overnight, the solution was cooled to 0° C and water (35 mL) was added. Stirring continued for one hour and then the mixture was diluted with dichloromethane and washed with sodium bicarbonate, water and brine. The organic layer was dried with sodium sulfate and evaporated under reduced pressure to remove the benzaldehyde. The residue was redissolved in dichloromethane and extracted with 2 M NaOH to remove traces of benzoic acid. The organic layer was then washed with water and brine and evaporated. The compound was not characterized, but directly benzylated by dissolving in dimethylformamide (25 mL) and adding sodium hydride (650 mg, 80% dispersion in oil, 24.6 mmol). After stirring for thirty minutes, benzyl bromide (3 mL, 25.2 mmol) was added and the reaction stirred for 20 hours. Methanol was added to decompose the excess sodium hydride and the reaction diluted with dichloromethane and then washed with water, and brine. After solvent evaporation, column chromatography of the resulting clear oil (9:1 hexane:ethyl acetate) gave **85** (1.53 g, 76%) as a white solid, $[\alpha]_D$ -39.1° (*c* 1,

$CHCl_3$), R_f 0.52 (9:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.23-7.55 (m, 15 H, Ph), 5.60 (s, 1 H, $PhC\underline{H}O_2$), 4.93 (d, 2 H, J_{gem} 11.5 Hz, 2 $PhC\underline{H}_2$), 4.82, 4.77 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.52 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.36 (dd, 1 H, $J_{6a,6b}$ 10.5 Hz, $J_{5,6a}$ 5 Hz, H-6a), 3.95 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.82 (t, 1 H, $J_{3,4} = J_{4,5}$ 10 Hz, H-4), 3.64-3.77 (m, 2 H, H-3, H-6b), 3.57 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.47 (t, 1 H, $J_{1,2}$ $J_{2,3}$ 8 Hz, H-2), 3.43 (dt, 1 H, $J_{5,6a}$ 5 Hz, $J_{4,5} = J_{5,6b}$ 10 Hz, H-5), 1.55-1.75 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.62, 138.47, 137.42 (Ph quaternary), 128.94, 128.35, 128.31, 127.25, 128.12, 128.04, 127.71, 127.62, 126.05 (Ph methine), 104.21 (C-1), 101.16 ($Ph\underline{C}HO_2$), 82.22 (C-3), 81.58 (C-2), 80.97 (C-4), 75.36, 75.14 ($Ph\underline{C}H_2$), 70.66 ($O\underline{C}H_2CH_2$), 68.88 (C-6), 66.07 (C-5), 31.86, 29.82, 29.43, 29.27, 26.15, 22.69 (octyl CH_2), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{35}H_{44}O_6$ (560.74): C, 74.97; H, 7.91. Found: C, 74.95; H, 7.87.

Octyl 2,3,6 tri-O-benzyl-β-D-glucopyranoside **(86)**. Compound **85** (1.5 g, 2.68 mmol), sodium cyanoborohydride (1.85 g, 29.5 mmol) and methyl orange indicator were dissolved in 25 mL dry tetrahydrofuran containing crushed 3 Å molecular sieves (2 g). The solution was cooled to 0° C and then ethereal hydrogen chloride was added until the red color of the solution persisted. After one hour TLC indicated the reaction was complete and the reaction was quenched by the addition of sodium bicarbonate. The reaction was filtered, diluted with dichloromethane, washed with water and brine and then evaporated. Column chromatography of the resulting oil (4:1 hexane:ethyl acetate) gave **86** (1.32 g, 88%) as a colorless oil, $[\alpha]_D$ -22.6° (*c* 0.8, $CHCl_3$), R_f 0.44 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.43 (m, 15 H, Ph), 4.96, 4.93, 4.73, 4.71, 4.61, 4.58 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.41 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.94 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.77 (dd, 1 H, $J_{5,6}$ 4 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.69 (dd, 1 H, $J_{5,6b}$ 5 Hz, $J_{6a,6b}$ 10.5 Hz, H-6b), 3.37-3.63 (m, 5 H, H-2, H-3, H-4, H-5, $OC\underline{H}_2CH_2$), 2.53 (d, 1 H, $J_{2,2\text{-}OH}$ 2 Hz, 2-OH), 1.59-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.72, 138.54, 138.03 (Ph quaternary), 128.56, 128.44, 128.39, 128.16, 127.99, 127.91, 127.83, 127.73 (Ph methine), 103.75 (C-1), 84.12 (C-3), 81.78 (C-2), 75.28, 74.74 ($Ph\underline{C}H_2$), 74.07 (C-5), 73.71 ($Ph\underline{C}H_2$), 71.74 (C-4), 70.47 ($O\underline{C}H_2CH_2$), 70.47 (C-6), 31.87, 29.83, 29.45, 29.29, 26.23, 22.70 (octyl CH_2), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{35}H_{46}O_6$ (562.75): C, 74.70; H, 8.24. Found: C, 74.58; H, 8.52.

Octyl 2,3,6 tri-O-benzyl-4-deoxy-4-fluoro-β-D-galactopyranoside **(87)**. To a solution of **86** (1.0 g, 1.78 mmol), in 19:1 dichloromethane:pyridine (50 mL) at 0° C, was added dropwise in dichloromethane (2 mL), triflic anhydride (1.30 mL, 7.55 mmol). After stirring for 30 min TLC showed the starting material was gone and a new spot (R_f 0.63, 4:1 hexane:ethyl acetate) appeared. The reaction was then extracted with ice cold 5% hydrochloric acid and water, dried with sodium sulfate and evaporated to an orange liquid. The product was directly dissolved in dry tetrahydrofuran (9 mL), cooled to 0° C and tetrabutylammonium fluoride (9 mL of a 1.0 M solution in tetrahydrofuran) added. After stirring for 15 hours and warming to room temperature, the solvent was removed and the residue chromatographed (4:1 hexane:ethyl acetate) to give **87** (803 mg, 80%) as a colorless oil, $[\alpha]_D$ -9.0° (*c* 0.3, $CHCl_3$), R_f 0.40 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.40 (m, 15 H, Ph), 4.69-4.95 (m, 5 H, 4 $PhC\underline{H}_2$, H-4), 4.56 (s, 2 H, $PhC\underline{H}_2$), 4.37 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{H1,F}$ 1 Hz, H-1), 3.92 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.40-3.78 (m, 6 H, H-2, H-3, H-5, H-6, H-6, $OC\underline{H}_2CH_2$), 1.58-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.60, 137.98, 137.84 (Ph quaternary), 128.50, 128.44, 128.33, 128.14, 127.88, 127.83, 127.67 (Ph methine), 103.66 (C-1), 86.09 (d, 1 C, $J_{C4,F}$ 183.4 Hz, C-4), 79.04 (C-2), 79.03 (d, 1 C, $J_{C3,F}$

18.0 Hz, C-3), 75.37, 73.77, 72.49 ($\mathrm{Ph\underline{C}H_2}$), 72.16 (d, 1 C, $J_{C5,F}$ 18.2 Hz, C-5), 70.31 ($\mathrm{O\underline{C}H_2CH_2}$), 67.89 (d, 1 C, $J_{C6,F}$ 5.3 Hz, C-6), 31.86, 29.78, 29.44, 29.27, 26.17, 22.69 (octyl CH_2), and 14.11 (octyl CH_3). ^{19}F NMR ($CDCl_3$): δ -217.3 (dt, 1 F, $J_{H4,F}$ 49.4 Hz, J_{H5} = $J_{H3,F}$ 26.8 Hz, 4-F)

Anal. Calcd for $C_{35}H_{45}FO_5$ (580.74): C, 74.44; H, 8.03. Found: C, 74.70; H, 7.94.

Octyl 4-deoxy-4-fluoro-β-D-galactopyranoside **(25)**. Galactoside **87** (515 mg, 0.91 mmol), was hydrogenated in methanol (30 mL) with 5% palladium on carbon (250 mg) as described for the preparation of **21**. Chromatography (19:1 dichloromethane: methanol) gave the product **25** (228 mg, 85%) as a white solid, $[\alpha]_D$ -30.3^o (*c* 0.6, CH_3OH), R_f 0.10 (19:1 dichloromethane: methanol). ^{1}H NMR (CD_3OD): δ 4.65 (dd, 1 H, $J_{3,4}$ 2.5 Hz, $J_{H4,F}$ 51 Hz, H-4), 4.21 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.86 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $\mathrm{OC\underline{H_2}CH_2}$), 3.40-3.75 (m, 6 H, H-2, H-3, H-5, H-6a, H-6b, $\mathrm{OC\underline{H_2}CH_2}$), 1.55-1.70 (m, 2 H, $\mathrm{OCH_2C\underline{H_2}}$), 1.20-1.40 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.64 (C-1), 90.16 (d, 1 C, $J_{C4,F}$ 179.5 Hz, C-4), 75.08 (d, 1 C, $J_{C5,F}$ 18 Hz, C-5), 73.64 (d, 1 C, 18.3 Hz, C-3), 72.57 (C-2), 71.01 ($\mathrm{O\underline{C}H_2CH_2}$), 61.06 (d, 1 C, $J_{C6,F}$ 6 Hz, C-6), 32.98, 30.77, 30.53, 30.38, 27.05, 23.69 (octyl CH_2), and 14.41 (octyl CH_3). ^{19}F NMR (CD_3OD): δ -220.1 (dt, 1 F, $J_{H4,F}$ 51 Hz, $J_{H3,F}$ = $J_{H5,F}$ 26 Hz, F-4).

Anal. Calcd for $C_{14}H_{27}FO_5$ (294.37): C, 57.13; H, 9.25. Found: C, 57.36; H, 9.54.

Octyl 3,6-di-O-benzoyl-4-deoxy-4-fluoro-β-D-galactopyranoside **(89)**. A solution of compound **25** (101 mg, 0.34 mmol) and dibutyl tin oxide (178 mg, 0.68 mmol) were refluxed in dry benzene (30 mL) overnight as described for the preparation of **40**. The solution was cooled to room temperature and crushed 4 Å molecular sieves (500 mg) and benzoyl chloride (90 µL, 0.68 mmol) were added. After stirring for one hour, TLC indicated complete conversion of the starting material to two different products. The solvent was evaporated and reaction chromatographed (3:1 hexane:ethyl acetate), **89** (63 mg, 37%) and **88** (68 mg, 50%), both as white solids. The monoester was converted to **89** in 76% yield by dissolution of the purified **88** in dichloromethane and then addition of 1.2 equivalents of both pyridine and benzoyl chloride. The data below is for compound **89** only, support of structure **88** was the presence of 5 aromatic hydrogens and the large downfield shift of H-3 in the ^{1}H NMR. $[\alpha]_D$ + 9.6° (*c* 0.6, $CHCl_3$), R_f 0.54 (3:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.98-8.15 (m, 4 H, Ph), 4.70-7.62 (m, 6 H, Ph), 5.19 (ddd, 1 H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3 Hz, $J_{H3,F}$ 27 Hz, H-3), 5.02 (dd, 1 H, $J_{3,4}$ 2.5 Hz, $J_{H4,F}$ 50 Hz, H-4), 4.64 (ddd, 1 H, $J_{6a,6b}$ 11 Hz, $J_{5,6a}$ 6.5 Hz, $J_{H6a,F}$ 1 Hz, H-6a), 4.51 (dd, 1 H, $J_{6a,6b}$ 11 Hz, $J_{5,6b}$ 7.5 Hz, H-6b), 4.45 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{H1,F}$ 1 Hz, H-1), 3.90-4.12 (m, 3 H, H-2, H-5, $OC\underline{H}_2CH_2$), 3.59 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.41 (d, 1 H, $J_{2,2\text{-}OH}$ 2-OH), 1.62-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2). 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 166.17, 166.10 (C=O), 133.55, 133.38, 130.04, 129.76 (Ph methine), 129.55, 129.37, (Ph quaternary), 128.51 (Ph methine), 103.13 (C-1), 86.31 (d, 1 C, $J_{C4,F}$ 186 Hz, C-4), 73.45 (d, 1 C, $J_{C5,F}$ 18.8 Hz, C-5), 71.18 (d, 1 C, $J_{C3,F}$ 17.2 Hz, C-3), 70.60 ($O\underline{C}H_2CH_2$), 69.40 (C-2), 61.93 (d,1 C, $J_{C6,F}$ 5.7 Hz, C-6), 31.83, 29.61, 29.35, 29.25, 25.96, 22.67 (octyl CH_2), and 14.11 (octyl CH_3). ^{19}F NMR ($CDCl_3$): -216.4 (dddd, 1 F, $J_{H4,F}$ 50 Hz, $J_{H3,F}$ = $J_{H5,F}$ 27 Hz, $J_{H1,F}$ 1 Hz, F-4).

Anal. Calcd for $C_{28}H_{35}FO_7$ (502.59): C, 66.92; H, 7.02 Found: C, 66.92; H, 6.91.

Octyl 3,6-di-O-benzoyl-4-deoxy-4-fluoro-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside **(90)**. Alcohol **89** (139 mg, 0.28 mmol) and tetraethylammonium bromide (64 mg, 0.30 mmol) were fucosylated as described for compound **41** using 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.11 mmol). Column chromatography of the residue (dichloromethane) gave the disaccharide **90**

(218 mg, 86%) as oil, $[\alpha]_D$ - 84.0° (c 0.1, $CHCl_3$), R_f 0.44 (dichloromethane). ^1H NMR ($CDCl_3$): δ 8.00-8.10 (m, 4 H, Ph), 7.40-7.62 (m, 6 H, Ph), 5.42 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 5.39 (ddd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 2.5 Hz, $J_{H3,F}$ 27 Hz, H-3), 5.06 (dd, 1 H, $J_{3,4}$ 2.5 Hz, $J_{H4,F}$ 50.5 Hz, H-4), 4.94, 4.75 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.58-4.69 (m, 4 H, H-1, 3 $PhC\underline{H}_2$), 4.50 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10 Hz, H-2), 4.27-4.42 (m, 4 H, $PhC\underline{H}_2$, H-5, H-6a, H-6b), 3.84-4.08 (m, 4 H, H-5, $OC\underline{H}_2CH_2$, H-3, H-2'), 3.64 (d, 1 H, $J_{3'4'}$ 1.5 Hz, H-4'), 3.47 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.60-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl $C\underline{H}_2$), 1.13 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 166.05, 165.63 (C=O), 138.87, 138.59, 137.88, 133.69, 133.32 (Ph quaternary),129.96, 129.76 (Ph methine), 129.55, 129.10 (Ph quaternary), 128.73, 128.48, 128.30, 128.19, 128.08, 127.66, 127.60, 127.43, 127.37 (Ph methine), 101.95 (C-1), 97.22 (C-1'), 86.18 (d, 1 C, $J_{C4,F}$ 185 Hz, C-4), 79.41 (C-4'),77.90 (C-3'),75.76 (C-2'), 75.55 (d, 1 C, $J_{C5,F}$ 17 Hz, C-5), 74.84, 73.24, 72.64 ($PhC\underline{H}_2$), 70.91 (C-2), 70.74 (d, 1 C, $J_{C3,F}$ 18.0 Hz, C-3), 70.62 ($O\underline{C}H_2CH_2$), 66.69 (C-5'), 61.84 (d, 1 C, $J_{C6,F}$ 6 Hz, C-6), 31.85, 29.70, 29.43, 29.31, 26.19, 22.66 (octyl CH_2), 16.57 (C-6'), and 14.12 (octyl CH_3). ^{19}F NMR ($CDCl_3$): δ -217.1 (dt, 1 F, $J_{H4,F}$ 50.5 Hz, $J_{H3,F} = J_{H5,F}$ 27 Hz, F-4).

Anal. Calcd for $C_{48}H_{59}FO_9$ (799.00): C, 72.16; H, 7.44. Found: C, 72.15; H, 7.59.

Octyl 4-deoxy-4-fluoro-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(7)**. To a solution of protected disaccharide **90** (155 mg, 0.17 mmol) in methanol (10 mL), 10% palladium on carbon (100 mg) was added and the solution stirred under a flow of hydrogen overnight. After completion of the reaction the catalyst was filtered away and the solvent evaporated. The residue was redissolved in methanol (20 mL) and 1 M NaOH (1 mL) added and the reaction stirred overnight. The solution was neutralized with Amberlite IR 120 H (+) resin, evaporated and the product further purified as described for **2** to give the product **7** (64 mg, 86%) as a white solid. ^{1}H NMR (CD_3OD): δ 5.20 (d, 1 H, $J_{1'2'}$ 3 Hz, H-1'), 4.69 (dd, 1 H, $J_{H4,F}$ 50.5 Hz, $J_{3,4}$ 2.5 Hz, H-4), 4.38 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.30 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.88 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.54-3.86 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4'), 3.52 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.50-1.65 (m, 2 H, $OCH_2C\underline{H}_2$), 1.23-1.45 (10 H, octyl CH_2), 1.18 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 103.13 (C-1), 101.47 (C-1'), 90.26 (d, 1 C, $J_{C4,F}$ 181 Hz, C-4), 78.50 (C-2), 75.00 (d, 1 C, $J_{C5,F}$ 18 Hz, C-5), 74.39 (d, 1 C, $J_{C3,F}$ 18.5 Hz, C-3), 73.71 (C-3'), 71.66 (C-4'), 70.92 ($O\underline{C}H_2CH_2$), 70.48 (C-2'), 67.80 (C-5'), 61.00 (d, 1 C, $J_{C6,F}$ 5.5 Hz, C-6), 32.99, 30.92, 30.57, 30.38, 27.25, 23.67 (octyl CH_2), 16.75 (C-6'), and 14.40 (octyl CH_3). ^{19}F NMR (CD_3OD): δ -220.2 (dt, 1 F, $J_{H4,F}$ 50.5 Hz, $J_{H3,F} = J_{H5,F}$ 27.0 Hz, F-4). FABMS (Glycerol-HCl): *m/z* = 463 $[M+Na]^+$ and 441 $[M+H]^+$ ($C_{20}H_{37}FO_9$ requires *m/z* = 440).

1,2,3,4 tetra-O-acetyl-6-deoxy-6-fluoro-D-galactopyranose **(95)**. 6-Deoxy-6-fluoro-1,2:3,4-di-O-isopropylidene-D-galactopyranose[168], **94** (1.54 g, 5.88 mmol) was dissolved in dichloromethane (2 mL), 99% trifluoroacetic acid (5 mL) and water (500 μL). After stirring for one hour the reaction mixture was evaporated to dryness. The hydrolyzed product was not characterized, but instead was dissolved in pyridine (5 mL) and acetic anhydride (5 mL) and stirred overnight. The next day the reaction was cooled to 0° C and excess acetic anhydride destroyed with methanol. Evaporation of the solvent followed by chromatography (3:1 hexane:ethyl acetate) gave **95** (1.74 g, 84%) as a white foam containing both anomers (α:β = 1:1), R_f 0.20 (3:1 hexane:ethyl acetate). Partial ^{1}H NMR ($CDCl_3$): δ 6.4 (s, 1 H, H-1α), 5.74 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1β). ^{13}C NMR: δ 92.10 (C-1α), 89.60 (C-1β).

2,3,4 tri-O-acetyl-6-deoxy-6-fluoro-α-D-galactopyranosyl bromide **(96)**. Peracetate **95** (1.09 g, 3.1 mmol), was dissolved in dichloromethane (5 mL) and hydrobromic acid (33% in acetic acid, 10 mL) was added. The reaction was stirred for two hours and then evaporated to dryness, coevaporating with dry toluene. The crude product was purified by a rapid chromatographic separation (3:1 hexane:ethyl acetate) to give the product **96** (920 mg, 80%) as a crystalline solid, R_f 0.28 (6:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 6.72 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.58 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.43 (dd, 1 H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 5.08 (dd, 1 H, $J_{1,2}$ 4 Hz, $J_{2,3}$ 10.5 Hz, H-2), 4.33-4.60 (m, 3 H, H-5, H-6a, H-6b), 2.15, 2.10, 2.02 (s, 3 H, acetate CH_3). ^{13}C NMR ($CDCl_3$): δ 170.04, 169.79, 169.68 (acetate C=O), 87.99 (C-1), 80.28 (d, 1 C, $J_{C6,F}$ 174 Hz, C-6), 71.59 (d, 1 C, $J_{C5,F}$ 23.6 Hz, C-5), 67.96 (C-2), 67.77 (C-3), 67.04 (d, 1 C, $J_{C5,F}$ 5.4 Hz, C-4), 20.72, 20.56, 20.51 (acetate CH_3).

Octyl 2,3,4 tri-O-acetyl-6-deoxy-6-fluoro-β-D-galactopyranoside (**97**). Compound **96** (1.43 g, 3.86 mmol), was glycosylated as described for the conversion of **67** to **68**, using silver triflate (1.49 g, 5.8 mmol), collidine (378 μL, 3.0 mmol) and octanol (1.8 mL, 11.4 mmol). The reaction was complete after 90 minutes. Chromatography of the crude reaction mix (6:1 hexane:ethyl acetate) gave **97** (1.19 g, 73%) as an oil, $[\alpha]_D$ -10.1° (*c* 0.8, $CHCl_3$), R_f 0.36 (6:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 5.44 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, H-4), 5.22 (dd, 1 H, $J_{2,3}$ 10.5 Hz, $J_{1,2}$ 8 Hz, H-2), 5.03 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{2,3}$ 10.5 Hz, H-3), 4.51 (ddd, 1 H, $J_{5,6a}$ 6.5 Hz, $J_{6a,6b}$ 9.5 Hz, $J_{H6a,F}$ 46.5 Hz, H-6a), 4.48 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.43 (ddd, $J_{5,6b}$ 5.5 Hz, $J_{6a,6b}$ 10 Hz, $J_{H6b,F}$ 46.5 Hz, C-6b), 3.87-4.01 (m, 2 H, H-5, $OC\underline{H}_2CH_2$), 3.48 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.15, 2.08, 1.99 (s, 3 H, acetate CH_3), 1.50-1.65 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 13 H, octyl CH_2, H-6). 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 170.21 170.13, 169.36 (acetate C=O), 101.39 (C-1), 80.86 (d, 1 C, $J_{C6,F}$ 172 Hz, C-6), 71.56 (d, 1 C, $J_{C5,F}$ 23.5 Hz, C-5), 70.94 (C-3), 70.39 ($O\underline{C}H_2CH_2$), 69.00 (C-2),67.21 (d, 1 C, $J_{C4,F}$ 6 Hz, C-4), 31.81, 29.42, 29.29, 29.25, 25.82, 22.66 (octyl CH_2), 20.73, 20.62, 20.59 (acetate CH_3), and 14.10 (octyl CH_3). ^{19}F NMR ($CDCl_3$): δ -230.9 (dt, 1 F, $J_{H6,F}$ 46.5 Hz, $J_{H5,F}$ 12.5 Hz, F-6).

Anal. Calcd for $C_{20}H_{33}FO_8$ (420.48): C, 57.13; H, 7.91. Found: C, 56.85; H, 7.85.

Octyl 6-deoxy-6-fluoro-β-D-galactopyranoside (**26**). Galactoside **97** (1.06 g, 2.54 mmol), was dissolved in methanol (20 mL) and sodium methoxide (100 mg) added. After stirring for 2 hours, the solution was neutralized by the addition of prewashed Amberlite IR 120 (H+) resin. Evaporation of the solvent followed by chromatography (19:1 dichloromethane: methanol) gave **26** (673 mg, 90%) as an oil, $[\alpha]_D$ -13.1° (*c* 0.7, CH_3OH), R_f 0.11 (19:1 dichloromethane: methanol). ^1H NMR (CD_3OD): δ 4.58 (ddd, 1 H, $J_{5,6a}$ 5 Hz, $J_{6a,6b}$ 9 Hz, $J_{H6a,F}$ 46.5 Hz, H-6a), 4.43 (ddd, $J_{5,6b}$ 7 Hz, $J_{6a,6b}$ 9 Hz, $J_{H6b,F}$ 48 Hz, C-6b), 4.22 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{1,6F}$ 1 Hz, H-1), 3.85 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.71-3.82 (m, 2 H, H-4, H-5), 3.23-3.59 (m, 3 H, H-2, H-3, $OC\underline{H}_2CH_2$), 1.53-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 13 H, octyl CH_2, H-6), 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.91 (C-1), 83.65 (d, 1 C, $J_{C6,F}$ 167.5 Hz, C-6), 74.72 (C-3), 74.70 (d, 1 C, $J_{C5,F}$ 21.9 Hz, C-5), 72.40 (C-2), 70.94 ($O\underline{C}H_2CH_2$), 69.94 (d, 1 C, $J_{C4,F}$ 6 Hz, C-4), 32.98, 30.83, 30.52, 30.38, 27.08, 23.69 (octyl CH_2), and 14.40 (octyl CH_3). ^{19}F NMR (CD_3OD): δ -230.0 (ddd, 1 F, $J_{H6a,F}$ 46.5 Hz, $J_{H6b,F}$ 48 Hz, $J_{H5,F}$ 13.5 Hz, F-6).

Anal. Calcd for $C_{14}H_{27}FO_5$ (294.37): C, 57.13; H, 9.25. Found: C, 57.09; H, 9.56.

Octyl 3,4-benzylidene-6-deoxy-6-fluoro-β-D-galactopyranoside (**98**). Compound **26** (162 mg, 0.55 mmol) and benzaldehyde dimethyl acetal (250 μL, 1.65 mmol) were dissolved in acetonitrile (20 mL) and p-toluenesulfonic acid (5 mg) added. After stirring for 2 hours the reaction was neutralized with triethylamine, evaporated and chromatographed (3:1 hexane:ethyl acetate). Both possible diastereomers of **98** were present in a 1:1 ratio (179 mg, 85%) and were easily separable by chromatography. Only the faster moving diastereomer was fully characterized and used, $[\alpha]_D$ + 19.8° (*c* 0.5, $CHCl_3$), R_f 0.45 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.3-7.5 (m, 5 H, Ph), 6.15 (s, 1 H, $PhC\underline{H}O_2$), 4.75 (ddd, 1 H, $J_{5,6a}$ 7 Hz, $J_{6a,6b}$ 10 Hz, $J_{H6a,F}$ 48 Hz, H-6), 4.66 (ddd, $J_{5,6b}$ 4.5 Hz, $J_{6a,6b}$ 10 Hz, $J_{H6b,F}$ 46.5 Hz, C-6b), 4.46 (dd, 1 H, $J_{3,4}$ 5.5, $J_{2,3}$ 8 Hz, H-3), 4.26 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.13 (dd, 1 H, $J_{3,4}$ 5.5 Hz, $J_{4,5}$ 2 Hz, H-4), 4.03 (dddd, $J_{5,6a}$ 7 Hz, $J_{5,6b}$ 4.5 Hz, $J_{4,5}$ 2 Hz, $J_{5H,F}$ 14.5 Hz), 3.96 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.73 (t, 1 H, $J_{1,2}$ $J_{2,3}$ 8 Hz, H-2), 3.55 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.54 (br. s, 1 H, 2-OH), 1.61-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.32 (Ph quaternary), 129.26, 128.46, 126.20 (Ph methine), 103.65 (C-1), 102.08 ($PhC\underline{H}O_2$), 82.38 (d, 1 C, $J_{C6,F}$ 170.5 Hz, C-6), 79.66 (C-3), 73.02 (d, 1 C, $J_{C4,F}$ 7 Hz, C-4), 72.37 (d, 1 C, $J_{C5,F}$ 22 Hz, C-5), 71.06 (C-2), 70.33 ($O\underline{C}H_2CH_2$), 31.86, 29.65, 29.41, 29.26, 26.02, 22.69 (octyl CH_2), and 14.13 (octyl CH_3). ^{19}F NMR ($CDCl_3$): δ -228.0 (ddd, 1 F, $J_{H6a,F}$ 48 Hz, $J_{H6b,F}$ 46.5 Hz, $J_{H5,F}$ 14.5 Hz, F-6).

Anal. Calcd for $C_{21}H_{31}FO_5$ (382.48): C, 65.95; H, 8.17. Found: C, 65.76; H, 8.06.

Octyl 3,4-benzylidene-6-deoxy-6-fluoro-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside **(99)**. Alcohol **98** (111 mg, 0.29 mmol) and tetraethylammonium bromide (67 mg, 0.32 mmol) were fucosylated as described for compound **41** using 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.16 mmol). Column chromatography of the residue (9:1 hexane:ethyl acetate) gave the disaccharide **99** (208 mg, 90%) as oil $[\alpha]_D$ - 66.7° (*c* 0.7, $CHCl_3$), R_f 0.75 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.18-7.5 (m, 20 H Ph), 6.10 (s, 1 H, $PhC\underline{H}O_2$), 5.51 (d, 1 H, $J_{1'2'}$ 4 Hz, H-1'), 5.00, 4.89 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.57-4.83 (m, 7 H, 4 $PhC\underline{H}_2$, H-6a, H-6b, H-3), 4.43 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.28 (q, 1 H, $J_{5',6'}$

6.5 Hz, H-5'), 4.15 (dd, 1 H, $J_{3,4}$ 5.5 Hz, $J_{4,5}$ 2 Hz, H-4), 4.09 (dd, 1 H, $J_{2'3'}$ 10.5 Hz, $J_{1'2'}$ 4 Hz, H-2'), 3.88-4.04 (m, 4 H, $OC\underline{H}_2CH_2$, H-5, H-2, H-3'), 3.63 (d, 1 H, $J_{3'4'}$ 2 Hz, H-4'), 3.45 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.50-1.60 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2), 1.13 (d, 1 H, $J_{5',6'}$ 6.5 Hz, H-6'), 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.99, 138.73, 138.33, 138.14 (Ph quaternary), 129.26, 128.37, 128.37, 128.33, 128.22, 128.13, 128.10, 127.65, 127.46, 127.35, 126.27 (Ph methine), 103.54 (C-1), 100.81 ($PhC\underline{H}O_2$), 96.36 (C-1'), 82.40 (d, 1 C, $J_{C6,F}$ 170.5 Hz, C-6), 81.36 (C-3), 79.48 (C-2), 78.00 (C-4'), 76.22 (C-3'), 74.75 ($Ph\underline{C}H_2$), 73.35 (d, 1 C, $J_{C4,F}$ 6.8 Hz, C-4), 73.34, 73.23 ($Ph\underline{C}H_2$), 72.90 (C-2'), 72.12 (d, 1 C, $J_{C5,F}$ 21.9 Hz, C-5), 69.69 ($O\underline{C}H_2CH_2$), 66.48 (C-5'), 31.83, 29.74, 29.42, 29.31, 26.18, 22.64 (octyl CH_2), 16.55 (C-6'), and 14.09 (octyl CH_3). ^{19}F NMR ($CDCl_3$): δ -228.5 (dt, 1 F, $J_{H6,F}$ 46.5 Hz, $J_{H5,F}$ 15 Hz, F-6).

Anal. Calcd for $C_{48}H_{59}FO_9$ (799.00): C, 72.16; H, 7.44. Found: C, 72.08; H, 7.56.

Octyl 6-deoxy-6-fluoro-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside (**8**). The protected disaccharide **99** (97 mg, 0.12 mmol) was dissolved in methanol (10 mL) and 10% palladium on carbon (50 mg) added and the solution stirred under a flow of hydrogen overnight. After completion of the reaction the catalyst was filtered away, and the product purified as described for **2** to give the product **8** (48 mg, 90%) as a white solid. ^1H NMR (CD_3OD): δ 5.18 (br. s, 1 H, H-1'), 4.54 (m, 2 H, H-6a, H-6b), 4.34 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.28 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.85 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.58-3.82 (m, 7 H, H-2, H-3, H-4, H-5, H-2', H-3', H-4'), 3.51 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.52-1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.22-1.43 (10 H, octyl CH_2), 1.18 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 103.43 (C-1), 101.56 (d, 1 C, $J_{C1',F}$ 4 Hz, C-1'), 83.59 (d, 1 C, $J_{C6,F}$ 167.5 Hz, C-6), 78.82 (C-2), 75.59 (d, 1 C,

$J_{C5,F}$ 22 Hz, C-5), 75.37 (C-3), 73.71 (C-3'), 71.72 (C-4'), 70.85 ($O\underline{C}H_2CH_2$), 70.57 (C-2'), 70.01 (d, 1 C, $J_{C4,F}$ 6 Hz, C-4), 67.78 (C-5'), 32.98, 30.96, 30.55, 30.37, 27.24, 23.66 (octyl CH_2), 16.76 (C-6'), and 14.40 (octyl CH_3).^{19}F NMR (CD_3OD): δ -232.3 (dt, 1 F, $J_{H6,F}$ 47.5 Hz, $J_{H5,F}$ 13.5 Hz, F-6). FABMS (Glycerol-HCl): *m/z* = 463 $[M+Na]^+$ and 441 $[M+H]^+$ ($C_{20}H_{37}FO_9$ requires *m/z* = 440).

Octyl 2,4,6-tri-O-benzyl-3-O-methyl-β-D-galactopyranoside **(100)**. To a solution of octyl 2,4,6-tri-O-benzyl-β-D-galactopyranoside (**46**, 527 mg, 0.94 mmol) in dry dimethylformamide (10 mL), sodium hydride (85 mg, 80% in oil, 2.83 mmol) was added. After stirring for thirty minutes, methyl iodide (175 µL, 2.81 mmol) was added and stirring continued for 2 hours overnight. The mixture was diluted with dichloromethane and washed with bicarbonate, water and brine. Chromatography (6:1 hexane:ethyl acetate) gave the product **100** (506 mg, 94%) as an oil, $[\alpha]_D$ - 9.1° (*c* 0.6, $CHCl_3$), R_f 0.37 (6:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.40 (m, 15 H, Ph), 4.90, 4.90, 4.74, 4.60, 4.45, 4.40 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.25 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.86-3.96 (m, 2 H, H-4, $OC\underline{H}_2CH_2$), 3.70 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, H-2), 3.49-3.61 (m, 3 H, H-5, H-6a, H-6b), 3.50 (s, 3 H, $OC\underline{H}_3$), 3.47 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.24 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3 Hz, H-3), 1.56-1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 139.03, 138.75, 138.01 (Ph quaternary), 128.43, 128.29, 128.24, 128.15, 127.98, 127.89, 127.77, 127.49, 127.46 (Ph methine), 103.94 (C-1), 84.56 (C-3), 79.54 (C-2), 75.07, 74.42, 73.57 ($Ph\underline{C}H_2$), 73.37 (C-5), 72.67 (C-4), 70.05 ($O\underline{C}H_2CH_2$), 68.94 (C-6), 58.86 (OCH_3), 31.85, 29.75, 29.46, 29.27, 26.18, 22.68 (octyl CH_2), and 14.11 (octyl CH_3).

Anal. Calcd for $C_{36}H_{48}O_6$ (576.78): C, 74.97; H, 8.39. Found: C, 74.82; H, 8.25.

Octyl 3-O-methyl-β-D-galactopyranoside **(27)**. Compound **100** (444 mg, 0.77 mmol), was dissolved in methanol (20 mL), 5% palladium on carbon (200 mg) added, and the reaction was allowed under a flow of hydrogen for 7 hours. The catalyst was filtered away, the solvent evaporated and the residue chromatographed (19:1 dichloromethane:methanol) to give **27** (201 mg, 85%) as a white solid $[\alpha]_D$ - 14.5° (*c* 0.4, CH_3OH), R_f 0.20 (19:1 dichloromethane:methanol). ^1H NMR (CD_3OD): δ 4.14 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.00 (d, 1 H, $J_{3,4}$ 4 Hz, H-4), 3.81 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.60-3.70 (m, 2 H, H-6a, H-6b), 3.34-3.53 (m 6 H, H-5, H-2, $OC\underline{H}_2CH_2$, $OC\underline{H}_3$), 3.05 (dd, 1 H, $J_{3,4}$ 4 Hz, $J_{2,3}$ 9.5 Hz, H-3), 1.46-1.61 (m, 2 H, $OCH_2C\underline{H}_2$), 1.12-1.40 (10 H, octyl CH_2), and 0.83 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.90 (C-1), 84.56 (C-3), 76.43 (C-5), 71.52 (C-2), 70.81 ($O\underline{C}H_2CH_2$), 65.98 (C-4), 62.47 (C-6), 57.31 ($O\underline{C}H_3$), 33.00, 30.82, 30.56, 30.40, 27.11, 23.70 (octyl CH_2), and 14.41 (octyl CH_3).

Anal. Calcd for $C_{15}H_{30}O_6$ (306.40): C, 58.80; H, 9.87. Found: C, 58.70; H, 9.78.

Octyl 4,6-benzylidene-3-O-methyl-β-D-galactopyranoside **(101)**. Galactoside **27** (98 mg, 0.32 mmol) was dissolved in acetonitrile (10 mL). Dimethoxytoluene (50 μL, 0.33 mmol) and toluenesulfonic acid (10 mg) were added and the solution stirred for eight hours. The reaction was quenched with triethylamine, the mixture diluted with dichloromethane and then washed with water and brine. Column chromatography of the resulting clear oil (1:1 hexane:ethyl acetate) gave **101** (90 mg, 72%) as a white solid, $[\alpha]_D$ + 6.5° (*c* 0.5, $CHCl_3$), R_f 0.50 (1:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.28-7.55 (m, 5 H, Ph), 5.54 (s, 1 H, $PhC\underline{H}O_2$), 4.26-4.38 (m, 3 H, H-1, H-4, H-6a), 4.08 (dd, 2 H, $J_{5,6}$ 2 Hz, $J_{6a,6b}$ 12 Hz, H-6b), 3.88-4.01 (m, 2 H, H-2, $OC\underline{H}_2CH_2$), 3.45-3.56 (m, 4 H, $OC\underline{H}_3$, $OC\underline{H}_2CH_2$), 3.41 (br. s, 1 H, H-5), 3.31 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3), 2.48 (d 1 H, $J_{2,2\text{-}OH}$ 2 Hz, 2-OH), 1.58-1.72 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 137.76 (Ph quaternary), 128.94, 128.12, 126.45 (Ph methine), 102.97 (C-1), 101.26 ($Ph\underline{C}HO_2$), 81.18 (C-3), 72.41 (C-4), 70.09 (C-2), 69.91 ($O\underline{C}H_2CH_2$), 69.40 (C-6), 66.70 (C-5), 57.23 ($O\underline{C}H_3$), 31.81, 29.54, 29.40, 29.22, 25.97, 22.65 (octyl CH_2), and 14.10 (octyl CH_3).

Anal. Calcd for $C_{22}H_{34}O_6$ (394.51): C, 66.98; H, 8.69. Found: C, 67.16; H, 8.84.

Octyl 4,6-benzylidene-3-O-methyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**102**). Alcohol **101** (114 mg, 0.29 mmol) and tetraethylammonium bromide (67 mg, 0.32 mmol) were fucosylated as described for compound **41** using 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.56 mmol). Column chromatography of the residue (9:1 hexane:ethyl acetate) gave the disaccharide **102** (208 mg, 90%) Column chromatography of the mixture (3:2 hexane:ethyl acetate) gave the disaccharide **102** (180 mg, 77%) as a white solid ($[\alpha]_D$ - 72.5° (*c* 0.4, $CHCl_3$), R_f 0.53 (3:2 hexane:ethyl acetate).^{1}H NMR ($CDCl_3$): δ 7.18-7.56 (m, 20 H Ph), 5.47-5.55 (m, 2 H, H-1', $PhC\underline{H}O_2$), 4.96, 4.88, 4.84, 4.74, 4.74, 4.65 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.43 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.41 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 4.23-4.33 (m, 2 H, H-4, H-6a), 3.94-4.12 (m, 4 H, H-2', H-3', H-2, H-6b),3.91 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.66 (br. s, 1 H, H-4'), 3.54 (dd, 1 H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 3.35-3.44 (m, 4 H, $OC\underline{H}_3$, $OC\underline{H}_2CH_2$), 3.33 (br. s, 1 H, H-5), 1.48-1.61 (m, 2 H, $OCH_2C\underline{H}_2$), 1.17-1.37 (10 H, octyl CH_2), 1.12 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 139.01, 138.81, 138.68, 137.68 (Ph quaternary),128.81, 128.20, 128.11, 128.02, 127.67, 127.31, 127.25, 126.40 (Ph methine), 101.70 (C-1), 101.16 ($Ph\underline{C}HO_2$), 97.06 (C-1'), 82.98 (C-3), 79.48 (C-4'), 78.03 (C-3'), 76.19 (C-3), 74.61, 72.99, 72.48 ($Ph\underline{C}H_2$), 71.84 (C-2),69.28 (C-6, $O\underline{C}H_2CH_2$), 66.30 (C-5'), 66.04 (C-5), 56.18 ($O\underline{C}H_3$), 31.77, 29.58, 29.43, 29.24, 26.17, 22.57 (octyl CH_2), 16.55 (C-6'), and 14.04 (octyl CH_3).

Anal. Calcd for $C_{49}H_{62}O_{10}$ (811.03): C, 72.57; H, 7.70. Found: C, 72.40; H, 7.79.

Octyl 3-O-methyl-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(9)**. The protected disaccharide **102** (172 mg, 0.21 mmol) was dissolved in ethanol (30 mL), 5% palladium on carbon (80 mg) added and the solution stirred under a flow of hydrogen overnight. After completion of the reaction the catalyst was filtered away, and the product purified as described for **2** to give the product **9** (59 mg, 61%) as a white solid. ^{1}H NMR (CD_3OD): δ 5.15 (d, 1 H, $J_{1'2'}$ 2.5 Hz, H-1'), 4.28 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5), 4.25 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.99 (d, 1 H, $J_{3,4}$ 3 Hz, H-4), 3.82 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.59-3.72 (m, 5 H, H-5, H-6a, H-6b, H-2', H-3'), 3.55 (br. s, 1 H, H-4'), 3.34-3.45 (m, 5 H, H-2, $OC\underline{H}_2CH_2$, $OC\underline{H}_3$), 3.31 (dd, 1 H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3 Hz, H-3), 1.42-1.56 (m, 2 H, $OCH_2C\underline{H}_2$), 1.15-1.35 (10 H, octyl CH_2), 1.08 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.81 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 103.44 (C-1), 100.58 (C-1'), 85.72 (C-3), 76.29 (C-3'), 75.16 (C-5), 73.81 (C-2), 71.80 (C-4'), 70.62 ($O\underline{C}H_2CH_2$), 70.30 (C-2'), 67.45 (C-4), 65.79 (C-5'), 62.41 (C-6), 56.83 ($O\underline{C}H_3$), 32.99, 30.98, 30.61, 30.41, 27.37, 23.63 (octyl CH_2), 16.72 (C-6'), and 14.41 (octyl CH_3). FABMS (Cleland): *m/z* = 475 $[M+Na]^+$ and 453 $[M+H]^+$ ($C_{21}H_{40}O_{10}$ requires *m/z* = 452).

Octyl 2-O-allyl-3,6-di-O-benzyl-4-O-methyl-β-D-galactopyranoside **(103)**. To a solution of octyl 2-O-allyl-3,6-di-O-benzyl-β-D-galactopyranoside (**52**, 192 mg, 0.38 mmol) in dry dimethylformamide (10 mL), sodium hydride (33 mg, 80% in oil, 1.1 mmol) was added. After stirring for thirty minutes, methyl iodide (50 µL, 0.80 mmol) was added and stirring continued for 2 hours. At this point, more sodium hydride (11 mg) and methyl iodide (25 µL) was added and the reaction stirred another 5 hours. The mixture was diluted with dichloromethane and washed with bicarbonate, water and brine. Chromatography (3:1 hexane:ethyl acetate) gave the product **103** (181 mg, 92%) as an oil, $[\alpha]_D$ - 19.3° (*c* 1, $CHCl_3$), R_f 0.60 (3:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.28-7.42 (m, 10 H, Ph), 5.96 (1 H, H_c allyl), 5.27 (1 H, H_a allyl), 5.14 (1 H, H_b allyl), 4.78, 4.71, 4.58, 4.52 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.38 (1 H,

H_d allyl), 4.25 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.22 (1 H, H_e allyl), 3.88 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.71 (dd, 1H, $J_{1,2}$ 7.5 Hz $J_{2,3}$ 9.5 Hz, H-2), 3.47-3.65 (4 H, H-4, H-5, H-6a, H-6b), 3.55 (s, 3 H, OCH_3), 3.37-3.47 (m, 2 H, H-3, $OC\underline{H}_2CH_2$), 1.52-1.65 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.67, 138.08 (Ph quaternary), 135.43 ($CH_2{=}\underline{C}HCH_2O$), 128.46, 128.35, 127.87, 127.81, 127.61 (Ph methine), 116.53 ($\underline{C}H_2{=}CHCH_2O$), 103.80 (C-1), 81.76 (C-3), 79.39 (C-2), 76.26 (C-5), 74.00 ($CH_2{=}CH\underline{C}H_2O$), 73.65 ($Ph\underline{C}H_2$), 73.15 (C-4), 73.02 ($PhC\underline{H}_2$), 69.88 ($O\underline{C}H_2CH_2$), 68.55 (C-6), 61.30 ($OCH_3$), 31.84, 29.69, 29.40, 29.27, 26.10, 22.67 (octyl CH_2), and 14.10 (octyl CH_3).

Anal. Calcd for $C_{32}H_{46}O_6$ (526.72): C, 72.97; H, 8.80. Found: C, 72.90; H, 8.77.

Octyl 3,6-di-O-benzyl-4-O-methyl-β-D-galactopyranoside **(104)**. To a solution of **103** (118 mg, 0.22 mmol) dissolved in 7:3:1 ethanol:benzene:water (15 mL), tris(triphenylphosphine) rhodium (I) chloride (30 mg, 0.03 mmol) and 1,4 diazabicyclo[2.2.2]octane (11 mg, 0.096 mmol) were added and the solution refluxed for 23 hours. The solvent was evaporated and the residue dissolved in 9:1 acetone water (15 mL). Mercuric oxide (5 mg) and mercuric chloride (900 mg) were added and stirring continued for six hours. The reaction was then diluted with dichloromethane and washed with saturated potassium iodide, water and brine. Evaporation of the organic layer followed by chromatography (3:1 hexane:ethyl acetate) gave **104** (159 mg, 86%) as an oil, $[\alpha]_D$ - 6.3° (*c* 0.3, $CHCl_3$), R_f 0.35 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.25-7.40 (m, 10 H, Ph), 4.78, 4.71, 4.58, 4.53 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.20 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.80-3.90 (m, 2 H, H-6a, $OC\underline{H}_2CH_2$), 3.73 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.5 Hz, H-2), 3.66 (d, 1 H, $J_{3,4}$ 3 Hz, H-4), 3.55-3.65 (m, 2 H, H-6b, H-5), 3.54 (s, 3 H, OCH_3), 3.47 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.40 (d, 1 H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3 Hz, H-3), 2.40 (d, 1 H, $J_{2,2\text{-}OH}$ 1.5 Hz, 2-OH), 1.54-1.67 (m, 2 H, OCH$_2$C$\underline{H}_2$), 1.20-1.37 (10 H, octyl CH$_2$), and 0.88 (t, 3 H, J$_{vic}$ 7 Hz, octyl CH$_3$). ^{13}C NMR (CDCl$_3$): δ 138.23, 137.98 (Ph quaternary), 128.48, 128.46, 127.87, 127.83, 127.78, 127.68 (Ph methine), 103.14 (C-1), 81.80 (C-3), 75.48 (C-5), 73.65 (PhC$\underline{H}_2$), 73.56 (C-4), 72.39 (PhC$\underline{H}_2$), 71.44 (C-2), 69.85 (O$\underline{C}$H$_2$CH$_2$), 68.46 (C-6), 61.22 (OCH$_3$), 31.81, 29.59, 29.39, 29.22, 25.99, 22.65 (octyl CH$_2$), and 14.08 (octyl CH$_3$).

Anal. Calcd for $C_{29}H_{41}O_6$ (486.65): C, 71.57; H, 8.70. Found: C, 71.41; H, 8.66.

Octyl 4-O-methyl-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside (**10**). Alcohol **104** (101 mg, 0.21 mmol) was fucosylated as described for **41** with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.408 mmol) and tetraethylammonium bromide (51 mg, 0.24 mmol) . At this point it was not possible to obtain a pure product, therefore the partially purified product, **105**, obtained after chromatography (3:1 hexane:ethyl acetate), was dissolved in methanol (10 mL), 5% palladium on carbon (50 mg) added and the solution stirred under a flow of hydrogen overnight. Final purification as described for **2** gave **10** (55 mg, 30%) as a white solid. ^{1}H NMR (CD$_3$OD): δ 5.19 (d, 1 H, J$_{1'2'}$ 3 Hz, H-1'), 4.28 (d, 1 H, J$_{1,2}$ 7.5 Hz, H-1), 4.28 (q, 1 H, J$_{5'6'}$ 6.5 Hz, H-5'), 3.86 (dt, 1 H, J$_{gem}$ 10 Hz, J$_{vic}$ 7 Hz, OC$\underline{H}_2$CH$_2$), 3.62-3.80 (m, 6 H, H-3', H-2', H-4, H-6a, H-6b, H-5), 3.60 (dd, 1 H, J$_{1,2}$ 7.5 Hz, J$_{2,3}$ 9.5 Hz, H-2), 3.54 (s, 3 H, OCH$_3$), 3.44-3.53 (m, 3 H, H-3, H-4', OC$\underline{H}_2$CH$_2$), 1.52-1.62 (m, 2 H, OCH$_2$C$\underline{H}_2$), 1.25-1.45 (10 H, octyl CH$_2$), 1.19 (d, 3 H, J$_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J$_{vic}$ 7 Hz, octyl CH$_3$). ^{13}C NMR (CD$_3$OD): δ 103.51 (C-1), 101.60 (C-1'), 80.21 (C-4), 79.31 (C-2), 76.45 (C-5), 73.75 (C-4'), 72.84 (C-3), 71.75 (C-3'), 70.82 (O$\underline{C}$H$_2$CH$_2$), 70.61 (C-2'), 67.77 (C-5'), 61.94 (OCH$_3$), 61.65 (C-6), 32.99, 30.96, 30.59, 30.39, 27.27, 23.68 (octyl CH$_2$), 16.77 (C-6'), and 14.40 (octyl CH$_3$).FABMS (Cleland): *m/z* = 475 [M+Na]$^+$ and 453 [M+H]$^+$ ($C_{21}H_{40}O_{10}$ requires *m/z* = 452).

Octyl 2-O-allyl-3,4-di-O-benzyl-β-D-galactopyranoside **(57)**. To a solution of octyl 2-O-allyl-3-O-benzyl-4,6-benzylidene-β-D-galactopyranoside (**51**, 2.07 g, 4.05 mmol) in 1:1 dichloromethane:ether (80 mL), was added lithium aluminum hydride (465 mg, 12.24 mmol). The solution was heated to reflux and then aluminum trichloride (165 mg 12.39 mmol) in ether (25 mL) was added dropwise over 45 min. The reaction was complete after 90 min. and the reaction was quenched by the addition of ethyl acetate and then water. The solution was diluted with dichloromethane and extracted with water, bicarbonate and brine. Chromatography (3:1 hexane:ethyl acetate) gave the product **57** (1.57 g, 76%) as a solid, $[\alpha]_D$ - 10.2° (*c* 0.8, $CHCl_3$), R_f 0.18 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.25-7.42 (m, 10 H, Ph), 5.98 (1 H, H_c allyl), 5.29 (1 H, H_a allyl), 5.16 (1 H, H_b allyl), 4.94, 4.84, 4.73, 4.65 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.42 (1 H, H_d allyl), 4.28 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.25 (1 H, H_e allyl), 3.90 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.65-3.80 (m, 3 H, H-2, H-3, H-4), 3.40-3.51 (m, 3 H, H-6a, H-6b, $OC\underline{H}_2CH_2$), 3.34 (dt, 1 H, $J_{5,6}$ 6.5 Hz, $J_{4,5}$ 1 Hz, H-5), 1.52-1.67 (m, 2 H, $OCH_2C\underline{H}_2$), 1.48 (dd, 1 H, $J_{6a,6\text{-}OH}$ 10.5 Hz, $J_{6b,6\text{-}OH}$ 6 Hz, 6-OH), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.64, 138.36 (Ph quaternary), 135.37 ($CH_2{=}\underline{C}HCH_2O$), 128.72, 128.46, 127.96, 127.70, 127.66 (Ph methine), 104.09 (C-1), 82.23 (C-3), 79.49 (C-2), 74.52 (C-4), 74.11 ($Ph\underline{C}H_2$), 74.03 ($Ph\underline{C}H_2$), 73.57 ($CH_2{=}CH\underline{C}H_2O$), 73.07 (C-5), 70.18 ($O\underline{C}H_2CH_2$), 62.11 (C-6), 31.85, 29.72, 29.42, 29.28, 26.09, 22.70 (octyl CH_2), and 14.12 (octyl CH_3).

Anal. Calcd for $C_{31}H_{44}O_6$ (512.69): C, 72.62; H, 8.65. Found: C, 72.50; H, 8.91.

Octyl 2-O-allyl-3,4-di-O-benzyl-6-O-methyl-β-D-galactopyranoside **(106)**. To a solution of **57** (219 mg, 0.43 mmol) in dry dimethylformamide (5 mL), sodium hydride (44 mg, 80% in oil, 1.46 mmol) was added. After stirring for 15 min., methyl iodide (80 µL, 1.28 mmol) was added and stirring continued overnight. The mixture was diluted with dichloromethane and washed with bicarbonate, water and brine. Chromatography (3:1 hexane:ethyl acetate) gave the product **106** (215 mg, 95%) as an oil, $[\alpha]_D$ + 2.3° (c 0.7, $CHCl_3$), R_f 0.59 (3:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.20-7.40 (m, 10 H, Ph), 5.97 (1 H, H_c allyl), 5.28 (1 H, H_a allyl), 5.15 (1 H, H_b allyl), 4.93, 4.78, 4.70, 4.64 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.41 (1 H, H_d allyl), 4.27 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.24 (1 H, H_e allyl), 3.90 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.82 (d, 1 H, $J_{3,4}$ 4 Hz, H-4), 3.68 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, H-2), 3.49-3.50 (m, 5 H, H-3, H-5, H-6a, H-6b, $OC\underline{H}_2CH_2$), 3.27 (s, 3 H, $OC\underline{H}_3$), 1.52-1.67 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.70 (Ph quaternary), 135.42 ($CH_2=\underline{C}HCH_2O$), 128.40, 128.34, 128.12, 127.52, 126.93 (Ph methine), 116.51 ($\underline{C}H_2=CHCH_2O$), 103.98 (C-1), 82.09 (C-3), 79.37 (C-2), 74.41, 73.95 ($Ph\underline{C}H_2$), 73.62 (C-5), 73.37 (C-4), 73.14 ($CH_2=CH\underline{C}H_2O$), 71.24 (C-6), 70.04 ($O\underline{C}H_2CH_2$), 59.06 ($O\underline{C}H_3$), 31.82, 29.66, 29.38, 29.25, 26.06, 22.66 (octyl CH_2), and 14.09 (octyl CH_3).

Anal. Calcd for $C_{32}H_{46}O_6$ (526.72): C, 72.97; H, 8.80. Found: C, 72.87; H, 8.68.

Octyl 3,4-di-O-benzyl-6-O-methyl-β-D-galactopyranoside (**107**). To a solution of **106** (173 mg, 0.33 mmol) dissolved in 7:3:1 ethanol:benzene:water (10 mL), tris(triphenylphosphine) rhodium (I) chloride (49 mg, 0.05 mmol) and 1,4 diazobicyclo[2.2.2]octane (19 mg, 0.14 mmol) were added and the solution refluxed for 20 hours. The solvent was evaporated and the residue dissolved in 9:1 acetone water (10 mL). Mercuric oxide (5 mg) and mercuric chloride (1.5 g) were added and stirring continued at room temperature overnight. The reaction was then diluted with dichloromethane and washed with saturated potassium iodide, water and brine. Evaporation of the organic layer, followed by chromatography (3:1 hexane:ethyl acetate) gave **107** (130 mg, 81%) as a white solid, $[\alpha]_D$ + 9.4° (c 0.9, $CHCl_3$), R_f 0.24

(3:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.24-7.40 (m, 10 H, Ph), 4.91, 4.75, 4.67, 4.65 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.22 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.94 (ddd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.5 Hz, $J_{2,2\text{-}OH}$ 2 Hz, H-2), 3.84-3.92 (m, 2 H, H-4, $OC\underline{H}_2CH_2$), 3.46-3.54 (m, 4 H, H-5, H-6a, H-6b, $OC\underline{H}_2CH_2$), 3.44 (dd, 1 H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3 Hz, H-3), 3.30 (s, 3 H, $OC\underline{H}_3$), 2.34 (d, 1 H, $J_{2,2\text{-}OH}$ 2 Hz, 2-OH), 1.57-1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.60, 138.24 (Ph quaternary), 128.54, 128.34, 128.22, 127.80, 127.68, 127.61 (Ph methine), 103.32 (C-1), 81.96 (C-3), 74.56 ($PhC\underline{H}_2$), 73.76 (C-5), 73.01 (C-4), 72.44 ($PhC\underline{H}_2$), 71.51 (C-2), 71.12 (C-6), 70.04 ($O\underline{C}H_2CH_2$), 59.14 ($O\underline{C}H_3$), 31.85, 29.59, 29.42, 29.24, 25.98, 22.68 (octyl CH_2), and 14.11 (octyl CH_3).

Anal. Calcd for $C_{29}H_{42}O_6$ (486.65): C, 71.57; H, 8.70. Found: C, 71.65; H, 8.66.

Octyl 3,4-di-O-benzyl-6-O-methyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**108**). Alcohol **107** (77 mg, 0.16 mmol) was fucosylated as described for **41** with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 0.635 mmol) and tetraethylammonium bromide (37 mg, 0.18 mmol). Column chromatography of the mixture (3:1 hexane:ethyl acetate) gave the disaccharide **108** (116 mg, 81%) as an oil $[\alpha]_D$ - 60.5° (*c* 0.2, $CHCl_3$), R_f 0.40 (3:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 6.96-7.40 (m, 20 H Ph), 5.71 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.95 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.40-4.88 (m, 11 H, H-1, H-5, 9 $PhC\underline{H}_2$), 4.24 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10 Hz, H-2), 4.03 (dd, 1 H, $J_{1'2'}$ 3.5 Hz, $J_{2'3'}$ 10 Hz, H-2'), 3.96 (dd, 1 H, $J_{2'3'}$ 10 Hz, $J_{3'4'}$ 2.5 Hz, H-3'), 3.92 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4), 3.89 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.74 (dd, $J_{2,3}$ 10 Hz, $J_{3,4}$ 2.5 Hz, H-3), 3.65 (br. s, 1 H, H-4'), 3.45-3.56 (m, 3 H, H-5, H-6a, H-6b), 3.38 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.23 (s, 3 H, $OC\underline{H}_3$), 1.45-1.58 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.35 (10 H, octyl CH_2), 1.12 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl

CH_3). ^{13}C NMR ($CDCl_3$): δ 139.01, 138.87, 138.44, 138.30, 138.06 (Ph quaternary),128.43, 128.37, 128.29, 128.25, 128.14, 128.03, 127.79, 127.55, 127.37, 127.25, 126.33 (Ph methine), 102.17 (C-1), 97.14 (C-1'), 84.55 (C-3), 79.70 (C-2), 78.20 (C-4'), 75.76 (C-3'), 74.76, 74.33 ($\mathrm{Ph\underline{C}H_2}$), 73.38 (C-4), 73.07, 72.62 ($\mathrm{Ph\underline{C}H_2}$), 72.09 (C-2'), 71.93 (C-5), 71.26 ($\mathrm{Ph\underline{C}H_2}$), 71.18 (C-6), 69.75 ($\mathrm{O\underline{C}H_2CH_2}$), 66.20 (C-5'), 59.13 ($\mathrm{O\underline{C}H_3}$), 31.88, 29.73, 29.55, 29.35, 26.31, 22.67 (octyl CH_2), 16.55 (C-6'), and 14.12 (octyl CH_3).

Anal. Calcd for $C_{56}H_{70}O_{10}$ (903.17): C, 74.47; H, 7.81. Found: C, 74.08; H, 7.84.

Octyl 6-O-methyl-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(11)**. The protected disaccharide **108** (80 mg, 0.09 mmol) was dissolved in methanol (5 mL), 5% palladium on carbon (20 mg) added and the solution stirred under a flow of hydrogen overnight. The catalyst was filtered away, and the product purified as described for **2** to give **11** (37 mg, 93%) as a white solid. ^1H NMR (CD_3OD): δ 5.19 (d, 1 H, $J_{1'2'}$ 2 Hz, H-1'), 4.31 (d, 1 H, $J_{1,2}$ 7 Hz, H-1), 4.29 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5), 3.85 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $\mathrm{OC\underline{H}_2CH_2}$), 3.70-3.80 (m, 3 H, H-3, H-4, H-2'), 3.55-3.70 (m, 6 H, H-2, H-3', H-4', H-5, H-6a, H-6b), 3.50 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $\mathrm{OC\underline{H}_2CH_2}$), 3.37 (s, 3 H, $\mathrm{OC\underline{H}_3}$), 1.53-1.65 (m, 2 H, $\mathrm{OCH_2C\underline{H}_2}$), 1.24-1.43 (10 H, octyl CH_2), 1.18 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 103.53 (C-1), 101.58 (C-1'), 79.00 (C-2), 75.62 (C-5), 74.77 (C-3), 73.74 (C-3'), 72.93 (C-6), 71.76 (C-4'), 70.83 ($\mathrm{O\underline{C}H_2CH_2}$), 70.62 (C-2'), 70.60 (C-4), 67.78 (C-5'), 59.45 ($\mathrm{O\underline{C}H_3}$), 33.00, 30.98, 30.58, 30.39, 27.28, 23.69 (octyl CH_2), 16.77 (C-6'), and 14.41 (octyl CH_3). FABMS (Cleland): $m/z = 491$ $[M+K]^+$, 475 $[M+Na]^+$ and 453 $[M+H]^+$ ($C_{21}H_{40}O_{10}$ requires $m/z = 452$).

Octyl 2,3,4,6-tetra-O-acetyl-β-D-gulopyranoside **(110)**. Silver triflate (609 mg, 2.37 mmol, dried in vacuo over phosphorus pentoxide for 1 hour), was stirred with collidine (155 µL, 1.27 mmol) and n-octanol (746 µL, 4.74 mmol) in dichloromethane (10 mL) containing crushed 3 Å molecular sieves (2.5 g) under nitrogen at -30° C for 20 minutes. To this solution was added dropwise, 2,3,4,6 tetra-O-acetyl α-D-gulopyranosyl bromide[170] (**109**, 650 mg, 1.58 mmol) in dichloromethane (5 mL). The reaction was stirred under nitrogen and warmed to room temperature. After stirring overnight the reaction was quenched with collidine (200 μL), filtered and evaporated. The residue was then chromatographed (4:1 hexane:ethyl acetate) to give the product **110** (478 mg, 66%) as an oil, $[\alpha]_D$ - 26.6° (*c* 0.7, $CHCl_3$), R_f 0.42 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 5.39 (t, 1 H, $J_{3,4} = J_{2,3}$ 3.5 Hz, H-3), 5.01 (dd, 1 H, $J_{2,3}$ 3.5 Hz, $J_{1,2}$ 8 Hz, H-2), 4.97 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, H-4), 4.75 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.13-4.30 (m, 3 H, H-5, H-6a, H-6b), 3.88, 3.50 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.15 (s, 6 H, acetate CH_3), 2.07, 2.02 (s, 3 H, acetate CH_3), 1.50-1.65 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 170.47, 169.60, 169.43, 168.94 (C=O), 98.61 (C-1), 70.35 (C-5), 70.06 ($O\underline{C}H_2CH_2$), 68.49 (C-3), 67.84 (C-4), 67.74 (C-2), 61.93 (C-6), 31.82, 29.52, 29.32, 29.26, 25.89, 22.66 (octyl CH_2), 20.74, 20.63 (acetate CH_3), and 14.09 (octyl CH_3).

Anal. Calcd for $C_{22}H_{36}O_{10}$ (460.52): C, 57.38; H, 7.88. Found: C, 57.52; H, 7.98.

Octyl β-D-gulopyranoside (**30**). Guloside **110** (373 mg, 0.81 mmol), was dissolved in methanol (10 mL) and sodium methoxide (60 mg) added. After stirring for 48 hours, the solution was neutralized by the addition of prewashed Amberlite IR 120 (H+) resin. The solvent was evaporated and the residue redissolved in water and then passed through a Sep-Pak then eluting first with water and then with methanol. The methanol eluant was evaporated, redissolved in water, filtered and lyophilized to give the product **30** (218 mg, 92%) as a gum, $[\alpha]_D$ - 60.9° (*c* 1, CH_3OH). ^1H NMR (CD_3OD): δ 4.57 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.94 (t, 1 H, $J_{2,3} = J_{3,4}$ 3.5 Hz, H-3), 3.82-3.92 (m, 2 H, H-4, $OC\underline{H}_2CH_2$), 3.64-3.77 (m, 3 H, H-5, H-6a, H-6b), 3.60

(dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 3.5 Hz, H-2), 3.51 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.55-1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.42 (10 H, octyl CH_2), and 0.89 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 102.32 (C-1), 74.80 (C-5), 73.13 (C-3), 71.18 (C-4), 70.60 ($O\underline{C}H_2CH_2$), 69.62 (C-2), 62.57 (C-6), 32.95, 30.83, 30.54, 30.35, 27.10, 23.66 (octyl CH_2), and 14.40 (octyl CH_3).

Anal. Calcd for $C_{14}H_{28}O_6$ (292.37): C, 57.51; H, 9.65. Found: C, 57.71; H, 9.62.

Octyl 2,3 isopropylidene-β-D-gulopyranoside **(112)**. Compound **30** (191 mg, 0.65 mmol), was dissolved in dimethylformamide (5 mL) and 2,2 dimethoxypropane (2.25 mL, 18.29 mmol) added. Toluenesulfonic acid (160 mg) was added and the reaction stirred for 1 hour. The reaction was then neutralized with triethylamine (500 μL), the solvent evaporated and the residue chromatographed (3:1 hexane:ethyl acetate) to give **111** (223 mg, 92%) as an oil, R_f 0.65 (3:1 hexane:ethyl acetate). ^1H NMR showed four isopropylidene methyl signals (1.50, 1.48, 1.42, 1.35), indicating the product to be the 2,3:4,6 di-O-isopropylidene derivative. The product was not further characterized but rather was dissolved in methanol (20 mL) then water (1 mL) and toluenesulfonic acid (10 mg) were added and the reaction stirred for 5 hours. The reaction was then neutralized with triethylamine (200 μL) and then the solvent evaporated and the residue chromatographed (3:1 hexane:ethyl acetate) to give **112** (147 mg, 74%) as an oil, $[\alpha]_D$ - 31.9° (*c* 0.3 , $CHCl_3$), R_f 0.30 (3:1 hexane:ethyl acetate).^1H NMR ($CDCl_3$): δ 4.84 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 4.38 (dd, 1 H, $J_{2,3}$ 6.5 Hz, $J_{3,4}$ 2 Hz, H-3), 4.26 (dd, 1 H, $J_{2,3}$ 6.5 Hz, $J_{1,2}$ 3 Hz, H-2), 4.02 (ddd, 1 H, $J_{4,5}$ 1 Hz, $J_{5,6a}$ 6 Hz, $J_{5,6b}$ 7 Hz, H-5), 3.93 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.77-3.93 (m 3 H, H-4, H-6a, H-6b), 3.51 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.20 (dd, 1 H, $J_{6,6\text{-}OH}$ 10 Hz, $J_{6,6\text{-}OH}$ 4 Hz, 6-OH), 1.55-1.65 (m, 3 H, 4-OH, $OCH_2C\underline{H}_2$), 1.50 (s, 3 H, $(C\underline{H}_3)_2CO_2$), 1.20-1.35 (10 H, octyl CH_2), 1.35 (s, 3 H, $(C\underline{H}_3)_2CO_2$), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ

109.32 ($(CH_3)_2\underline{C}O_2$), 99.12 (C-1), 74.85 (C-5), 73.40 (C-3), 71.99 (C-4), 69.48 ($O\underline{C}H_2CH_2$), 67.61 (C-2), 63.65 (C-6), 31.77, 29.46, 29.30, 29.15 (octyl CH_2), 26.78 ($(\underline{C}H_3)_2CO_2$), 25.94, (octyl CH_2), 24.59 ($(\underline{C}H_3)_2CO_2$), 22.60 (octyl CH_2), and 14.04 (octyl CH_3).

Anal. Calcd for $C_{17}H_{32}O_6$ (332.44): C, 61.42; H, 9.70. Found: C, 61.43; H, 9.96.

Octyl 4,6-di-O-benzyl-2,3-isopropylidene-β-D-gulopyranoside (**113**). Compound **112** (75.4 mg, 0.23 mmol) was dissolved in dry dimethylformamide (3 mL). Sodium hydride (45 mg, 80% dispersion in oil, 1.50 mmol) was added and the mixture stirred for 15 minutes. Benzyl bromide (162 μL, 1.36 mmol) was added and stirring continued for 15 hours. The solution was then cooled to 0° C, quenched with water, diluted with dichloromethane and washed with sodium bicarbonate, water and brine. Evaporation of the organic layer gave a brown liquid which was chromatographed (9:1 hexane:ethyl acetate) to give **113** (106 mg, 91%) as a colorless oil, $[\alpha]_D$ - 86.3° (c 0.5, $CHCl_3$), R_f 0.32 (9:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.22-7.47 (m, 10 H, Ph), 4.68, 4.54, 4.52, 4.46 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.40 (d, 1 H, $J_{1,2}$ 7 Hz, H-1), 4.32 (dd, 1 H, $J_{3,4}$ 2 Hz, $J_{2,3}$ 5.5 Hz, H-3), 4.00 (dd, 1 H, $J_{2,3}$ 5.5 Hz, $J_{1,2}$ 7 Hz, H-2), 3.81-3.95 (m, 2 H, H-5, $OC\underline{H}_2CH_2$), 3.74 (br. t, 1 H, $J_{4,5} = J_{3,4}$ 2 Hz, H-4), 3.70 (dd, 1 H, $J_{5,6a}$ 6.5 Hz, $J_{6a,6b}$ 10 Hz, H-6a), 3.62 (dd, 1 H, $J_{5,6b}$ 6 Hz, $J_{6a,6b}$ 10 Hz, H-6b), 3.48 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.54-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.48, 1.34 (s, 3 H, $(C\underline{H}_3)_2CO_2$), 1.20-1.40 (10 H, octyl CH_2), 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.25, 137.78 (Ph quaternary), 128.38, 128.13, 127.91, 127.63 (Ph methine), 109.28 ($(CH_3)_2\underline{C}O_2$), 102.60 (C-1), 74.97 (C-5), 74.42 (C-4), 73.48, 72.98 ($Ph\underline{C}H_2$), 72.92 (C-3), 72.84 (C-2), 69.69 ($O\underline{C}H_2CH_2$), 69.04 (C-6), 31.84, 29.61, 29.43, 29.25 (octyl CH_2), 28.08, 26.11 ($(\underline{C}H_3)_2CO_2$), 25.95, 22.67, (octyl CH_2), and 14.11 (octyl CH_3).

Anal. Calcd for $C_{31}H_{44}O_6$ (512.69): C, 72.63; H, 8.65. Found: C, 72.61; H, 8.74.

Octyl 4,6-di-O-benzyl-β-D-gulopyranoside (**114**). Compound **113** (320 mg, 0.63 mmol) was dissolved in dichloromethane (5 mL) and then water (500 μL) and 99% trifluoroacetic acid (2 mL) added. After stirring for 30 min, the reaction was evaporated to dryness and the product purified by chromatography (3:1 hexane:ethyl acetate) to give **114** (258 mg, 87%) as a white solid, $[\alpha]_D$ - 53.3° (*c* 0.5, $CHCl_3$), R_f 0.28 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.38 (m, 10 H, Ph), 4.42-4.64 (m, 5 H, $PhC\underline{H}_2$, H-1), 4.18 (t, 1 H, $J_{2,3} = J_{3,4}$ 3 Hz, H-3), 4.11 (dt, 1 H, $J_{4,5}$ 1.5 Hz, $J_{5,6a} = J_{5,6b}$ 6.5 Hz, H-5), 3.90 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.73 (dd, 1 H, $J_{2,3}$ 3 Hz, $J_{1,2}$ 8 Hz, H-2), 3.67 (dd, 1 H, $J_{5,6a}$ 6.5 Hz, $J_{6a,6b}$ 10 Hz, H-6a), 3.62 (dd, 1 H, $J_{5,6b}$ 6.5 Hz, $J_{6a,6b}$ 10 Hz, H-6b), 3.60 (dd, 1 H, $J_{4,5}$ 1.5 Hz, $J_{3,4}$ 3 Hz, H-4), 3.46 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.64, 2.50 (br. s, 1 H, 2-OH, 3-OH), 1.54-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.28, 137.98 (Ph quaternary), 128.36, 128.03, 127.83, 127.68, 127.61 (Ph methine), 100.27 (C-1), 75.77 (C-5), 73.43, 72.93 ($Ph\underline{C}H_2$), 72.37 (C-4), 69.81 (C-3), 69.65 ($O\underline{C}H_2CH_2$), 68.99 (C-2), 68.26 (C-6), 31.84, 29.67, 29.43, 29.25, 26.03, 22.67, (octyl CH_2), and 14.11 (octyl CH_3).

Anal. Calcd for $C_{28}H_{40}O_6$ (472.62): C, 71.16; H, 8.53. Found: C, 71.43; H, 8.69.

Octyl 3-O-acetyl-4,6-di-O-benzyl-β-D-gulopyranoside (**115**). Compound **114** (227 mg, 0.48 mmol) was dissolved in benzene (5 mL) and then triethylorthoformate (1.4 mL, 8.42 mmol) added followed by p-toluenesulfonic acid (5 mg). After stirring for 1 hour TLC indicated the presence of one carbohydrate spot (R_f 0.67, 3:1 hexane:ethyl acetate). The reaction was then neutralized with triethylamine (100 μL), diluted with ether and washed with water, bicarbonate and brine. After drying, the solvent was evaporated and the residue dissolved in 80% aqueous acetic acid (5 mL). The reaction was stirred for 10 min and then evaporated. The product was purified by chromatography (3:1 hexane:ethyl acetate) to give **115** (240 mg, 97%) as a clear oil, R_f 0.34 (3:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.00-7.20 (m, 10 H, Ph), 5.32 (t, 1 H, $J_{2,3} = J_{3,4}$ 3.5 Hz, H-3), 4.60 (d, 1 H, J_{vic} 11.5 Hz, PhC$\underline{H}_2$), 4.50 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.42, 4.40, 4.34 (d, 1 H, J_{vic} 11.5 Hz, PhC$\underline{H}_2$), 3.74-3.88 (m, 3 H, H-2, H-5, OC$\underline{H}_2$CH_2), 3.49-3.60 (m, 3 H, H-4, H-6a, H-6b), 3.48 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OC$\underline{H}_2$CH_2), 2.14, (d, 1 H, $J_{2,2\text{-}OH}$ 3.5 Hz, 2-OH), 2.06 (s, 3 H, acetate CH_3), 1.54-1.70 (m, 2 H, OCH_2C$\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).

Octyl 4,6-di-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-gulopyranoside (**117**). Alcohol **115** (200 mg, 0.39 mmol) was fucosylated as described for **41** with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.56 mmol) and tetraethylammonium bromide (90 mg, 0.43 mmol). At this point it was not possible to obtain **116** as a pure product, therefore the partially purified product (obtained after chromatography in 3:1 hexane:ethyl acetate, R_f 0.55) was dissolved in methanol (10 mL), and sodium methoxide (15 mg) added. The residue left after neutralization with Amberlite IR-120 H (+) resin and solvent evaporation was chromatographed (6:1 hexane:ethyl acetate) to give **117** (269 mg, 78%) as an oil, $[\alpha]_D$ - 95.3^o (*c* 0.2, $CHCl_3$), R_f 0.35 (3:1 hexane:ethyl acetate). ^{1}H-NMR ($CDCl_3$): δ 7.20-7.45 (m, 25 H, Ph), 4.98 (d, 1 H, J_{vic} 11.5 Hz, PhC$\underline{H}_2$), 4.42-4.84 (m, 11 H, H-1, H-1', 9 PhC$\underline{H}_2$), 4.20 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 4.09 (dt, 1 H, $J_{5,6a} = J_{5,6b}$ 6.5 Hz, $J_{4,5}$ 1.5 Hz, H-5), 4.04 (dd, 1 H, $J_{2',3'}$ 10 Hz, $J_{3',4'}$ 3.5 Hz, H-3'), 3.91-3.99 (m, 2 H, H-2', H-3), 3.86 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OC$\underline{H}_2$CH_2), 3.74 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 3.5 Hz, H-2), 3.58-3.70 (m, 4 H, H-4, H-4', H-6a, H-6b), 3.37 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OC$\underline{H}_2$CH_2), 1.46-1.58 (m, 2 H, OCH_2C$\underline{H}_2$), 1.18-1.35 (10 H, octyl CH_2), 1.11 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C-NMR

($\mathrm{CDCl_3}$): δ 138.70, 138.39, 138.22, 138.10 (Ph quaternary), 128.52, 128.42, 128.39, 128.35, 128.27, 128.24, 128.13, 127.96, 127.86, 127.66, 127.58, 127.39 (Ph methine), 98.98 (C-1), 94.74 (C-1'), 79.92 (C-2), 77.66 (C-4'), 76.12 (C-5), 75.63 (C-2'), 74.85, 74.36 ($\mathrm{Ph\underline{C}H_2}$), 73.37, 73.24, 72.85 ($\mathrm{Ph\underline{C}H_2}$), 71.99 (C-4), 69.09 ($\mathrm{O\underline{C}H_2CH_2}$), 69.04 (C-6), 66.83 (C-3), 65.52 (C-5'), 31.89, 29.84, 29.51, 29.38, 26.19, 22.70 (octyl $\mathrm{CH_2}$), 16.54 (C-6'), and 14.13 (octyl $\mathrm{CH_3}$).

Anal. Calcd for $\mathrm{C_{55}H_{68}O_{10}}$ (889.15): C, 74.30; H,7.71. Found: C, 74.49; H, 7.74.

Octyl 2-O-(α-L-fucopyranosyl)-β-D-gulopyranoside (**12**). The protected disaccharide **117** (87 mg, 0.098 mmol) was dissolved in methanol (5 mL), 5% palladium on carbon (20 mg) added and the solution stirred under a flow of hydrogen for 15 hours. The catalyst was filtered away, and the product purified as described for **2** to give **12** (39 mg, 91%) as a white solid. ^{1}H NMR ($\mathrm{D_2O}$): δ 5.01 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1'), 4.78 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.34 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 4.21 (t, 1 H, $J_{2,3} = J_{3,4}$ 3 Hz, H-3), 4.00 (t, 1 H $J_{5,6a} = J_{5,6b}$ 6.5 Hz, H-5), 3.92 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $\mathrm{OC\underline{H}_2CH_2}$), 3.72-3.91 (m, 6 H, H-4, H-6a, H-6b, H-2', H-3', H-4'), 3.70 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 3 Hz, H-2), 3.60 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $\mathrm{OC\underline{H}_2CH_2}$), 1.53-1.66 (m, 2 H, $\mathrm{OCH_2C\underline{H}_2}$), 1.24-1.50 (10 H, octyl $\mathrm{CH_2}$), 1.22 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.86 (t, 3 H, J_{vic} 7 Hz, octyl $\mathrm{CH_3}$). ^{13}C NMR ($\mathrm{CD_3OD}$): δ 100.55 (C-1), 97.24 (C-1'), 74.90 (C-5), 73.98 (C-3'), 73.79 (C-3), 71.70 (C-4'), 70.51 (C-4), 70.30 ($\mathrm{O\underline{C}H_2CH_2}$), 69.99 (C-2), 69.71 (C-2'), 67.66 (C-5'), 62.54 (C-6), 33.00, 31.02, 30.59, 30.45, 27.42, 23.68 (octyl $\mathrm{CH_2}$), 16.61 (C-6'), and 14.40 (octyl $\mathrm{CH_3}$). FABMS (Cleland): $m/z = 461$ $[\mathrm{M+Na}]^+$ and 439 $[\mathrm{M+H}]^+$ ($\mathrm{C_{20}H_{38}O_{10}}$ requires $m/z = 438$).

Octyl 2-O-allyl-4-O-benzoyl-3,6-di-O-benzyl-β-D-glucopyranoside (**80**). To a solution of octyl 2-O-allyl-3,6-di-O-benzyl-β-D-galactopyranoside (**52**, 926 mg, 1.81 mmol), in 19:1 dichloromethane:pyridine (40 mL) at 0° C, was added dropwise in dichloromethane (5 mL), triflic anhydride (1.30 mL, 7.68 mmol). After stirring for 45 min TLC showed the starting material was gone and a new spot (R_f 0.55, 4:1 hexane:ethyl acetate) appeared. The reaction was then extracted with ice cold 5% hydrochloric acid and water, dried with sodium sulfate and evaporated to an orange liquid. The product was directly dissolved in dry dimethylformamide (100 mL), cooled to 0^o C and sodium benzoate (2 g, 13.70 mmol) added. After stirring for 15 hours and warming to room temperature, the reaction was diluted with dichloromethane and washed with water, bicarbonate and brine, The solvent was evaporated and the residue chromatographed (39:1 toluene:ethyl acetate) to give **80** (817 mg, 73%) as an oil, $[\alpha]_D$ -55.7^o (*c* 0.3, $CHCl_3$), R_f 0.35 (39:1 toluene:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.00-8.00 (m, 15 H, Ph), 5.96 (1 H, H_c allyl), 5.30 (1 H, H_a allyl), 5.20 (t, 1 H, $J_{3,4}$ = $J_{4,5}$10 Hz, H-4), 5.18 (1 H, H_b allyl), 4.79, 4.62, 4.51, 4.46 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.43 (1 H, H_d allyl), 4.42 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.22 (1 H, H_e allyl), 3.96 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.69 (t, 1 H, $J_{3,4}$ = $J_{2,3}$ 10 Hz, H-3), 3.56-3.68 (m, 3 H, H-5, H-6a, H-6b), 3.54 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.43 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10 Hz, H-2), 1.58-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.45 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 165.42 (C=O), 138.08, 137.94 (Ph quaternary), 135.01 (CH_2=$\underline{C}HCH_2O$), 133.12, 129.79, 128.37, 128.24, 128.14, 128.06, 127.65, 127.51 (Ph methine), 117.01 ($\underline{C}H_2$=$CHCH_2O$), 103.54 (C-1), 81.80 (C-3), 81.44 (C-2), 75.07 ($Ph\underline{C}H_2$), 73.73 (C-5), 73.65 ($Ph\underline{C}H_2$), 71.51 (C-4), 70.30 ($O\underline{C}H_2CH_2$), 69.94 (C-6), 31.85, 29.73, 29.39, 29.28, 26.11, 22.68 (octyl CH_2), and 14.12 (octyl CH_3).

Anal. Calcd for $C_{38}H_{48}O_7$ (616.80): C, 74.00; H, 7.84. Found: C, 73.71; H, 8.13.

Octyl 4-O-benzoyl-3,6-di-O-benzyl-β-D-glucopyranoside **(118)**. To a solution of **80** (98 mg, 0.16 mmol) dissolved in 7:3:1 ethanol:benzene:water (10 mL), tris(triphenylphosphine) rhodium (I) chloride (21 mg, 0.02 mmol) and 1,4 diazobicyclo[2.2.2]octane (8 mg, 0.07 mmol) were added and the solution refluxed for 20 hours. The solvent was evaporated and the residue dissolved in 9:1 acetone water (15 mL). Mercuric oxide (2 mg) and mercuric chloride (500 mg) were added and stirring continued at room temperature overnight. The reaction was then diluted with dichloromethane and washed with saturated potassium iodide, water and brine. Evaporation of the organic layer followed by chromatography (4:1 hexane:ethyl acetate) gave **118** (65 mg, 72%) as an oil, $[\alpha]_D$ - 37.0° (*c* 0.7, $CHCl_3$), R_f 0.40 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.00-8.00 (m, 15 H, Ph), 5.26 (t, 1 H, $J_{3,4} = J_{4,5}$ 10 Hz, H-4), 4.78, 4.70 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.48 (s, 2 H, $PhC\underline{H}_2$), 4.36 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.95 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.59-3.77 (5 H, H-2, H-3, H-5, H-6a, H-6b), 3.56 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.41 (d, 1 H, $J_{2,2\text{-}OH}$ 2 Hz, 2-OH), 1.60-1.72 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 165.44 (C=O), 138.09, 137.88 (Ph quaternary), 133.23, 129.84, 129.73, 128.43, 128.28, 128.01, 127.71, 127.62, 127.56 (Ph methine), 102.74 (C-1), 81.25 (C-3), 74.59 (C-5), 74.30 ($PhC\underline{H}_2$), 74.04 (C-2), 73.67 ($PhC\underline{H}_2$), 71.26 (C-4), 70.37 ($O\underline{C}H_2CH_2$), 69.81 (C-6), 31.85, 29.66, 29.42, 29.27, 26.04, 22.69 (octyl CH_2), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{35}H_{44}O_7$ (576.73): C, 72.89; H, 7.69. Found: C, 72.74; H, 7.87.

Octyl 2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-3,6-di-O-benzyl-β-D-glucopyranoside **(120)**. Alcohol **118** (79 mg, 0.14 mmol) was fucosylated as described for **41** with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 0.683 mmol) and tetraethylammonium bromide (32 mg, 0.15 mmol). At this point it was not possible to obtain a pure product (**119**), therefore the partially purified product was dissolved in methanol (10 mL), and sodium methoxide (15 mg) added. Removal of the benzoate was sluggish and after stirring for 2 days at room temperature the solution was heated at 50 C for 12 hours to complete the saponification. The residue left after neutralization with Amberlite IR-120 H(+) resin and solvent evaporation was chromatographed (4:1 hexane:ethyl acetate) to give **120** (97 mg, 80%) as an oil, $[\alpha]_D$ -84.4° (*c* 0.3, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.38-7.90 (m, 25 H, Ph), 5.54 (d, 1 H, $J_{1'2'}$ = 3.5 Hz, H-1'), 4.50-5.00 (m, 10 H, $PhC\underline{H}_2$), 4.44 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.40 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 4.08 (dd, 1 H, $J_{2'3'}$ 10.5 Hz, $J_{1'2'}$ 3.5 Hz, H-2'), 3.98 (dd, 1 H, $J_{2'3'}$ 10.5 Hz, $J_{3'4'}$ 2.5 Hz, H-3'), 3.86 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.59-3.81 (m, 6 H, H-2, H-3, H-4, H-4', H-6a, H-6b), 3.36 -3.48 (m, 2 H, $OC\underline{H}_2CH_2$, H-5), 2.57 (d, 1 H, $J_{4,4\text{-}OH}$ 2 Hz, 4-OH), 1.45-1.60 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.35 (10 H, octyl CH_2), 1.11 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.83, 138.77, 138.75, 138.22, 137.81 (Ph quaternary), 128.49, 128.46, 128.35, 128.17, 128.13, 127.81, 127.75, 127.53, 127.46, 127.41, 127.11 (Ph methine), 101.93 (C-1), 97.33 (C-1'), 85.79 (C-3), 79.87 (C-2), 77.96 (C-4'), 75.87 (C-5), 74.96 (C-4), 74.80, 74.20, 73.74, 73.72 ($Ph\underline{C}H_2$), 73.64 (C-3'), 72.96 ($Ph\underline{C}H_2$), 72.64 (C-2'), 70.73 ($O\underline{C}H_2CH_2$), 69.96 (C-6), 66.37 (C-5'), 31.87, 29.77, 29.49, 29.34, 26.26, 22.67 (octyl CH_2), 16.63 (C-6'), and 14.11 (octyl CH_3).

Anal. Calcd for $C_{55}H_{68}O_{10}$ (889.14): C, 74.30; H, 7.71. Found: C, 74.01; H, 7.84.

Octyl 2-O-(α-L-fucopyranosyl)-β-D-glucopyranoside **(13)**. The protected disaccharide **120** (90 mg, 0.10 mmol) was dissolved in methanol (5 mL), 5% palladium on carbon (50 mg) added and the solution stirred under a flow of hydrogen for 24 hours. The catalyst was filtered away, and the product purified as described for **2** to give **13** (33 mg, 74%) as a white solid. ^1H NMR (CD_3OD): δ 5.22 (br. s, 1 H, H-1'), 4.35 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.29 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.91 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.85 (dd, 1 H, $J_{5,6}$ 2 Hz, $J_{6a,6b}$ 12 Hz, $H\text{-}6_a$), 3.74, (m, 1 H, H-2'), 3.53-3.70 (m, 4 H, H-4, H-3', H-4', H-6b), 3.50 (dt, 1 H,

J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.27-3.37 (m, 2 H, H-2, H-3), 1.50-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.23-1.45 (10 H, octyl CH_2), 1.18 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.89 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 103.06 (C-1), 101.44 (C-1'), 80.96 (C-2), 78.76 (C-3), 77.83 (C-5), 75.12 (C-4), 73.76 (C-3'), 71.74 (C-4'), 71.56 (C-3'), 70.85 ($O\underline{C}H_2CH_2$), 70.61 (C-2'), 67.81 (C-5'), 62.73 (C-6), 33.01, 30.96, 30.63, 30.41, 27.31, 23.70 (octyl CH_2), 16.77 (C-6'), and 14.40 (octyl CH_3). FABMS (Cleland): *m/z* = 477 $[M+K]^+$, 461 $[M+Na]^+$ and 439 $[M+H]^+$ ($C_{20}H_{38}O_{10}$ requires *m/z* = 438).

3-azido-3-deoxy-1:2,5:6-di-O-isopropylidene-α-D-galactofuranose **(122)**. To a solution of 1:2,5:6-di-O-isopropylidene-α-D-gulofuranose[169] (**121**, 1.03 g, 3.96 mmol), in 19:1 dichloromethane:pyridine (40 mL) at 0° C, was added dropwise in dichloromethane (2 mL), triflic anhydride (2.83 mL, 16.76 mmol). After stirring for 15 min., TLC showed the starting material was gone and a new spot (R_f 0.36, 3:1 hexane:ethyl acetate) had appeared. The reaction was then extracted with ice cold 5% hydrochloric acid and water, dried with sodium sulfate and evaporated to an orange liquid. This product was directly dissolved in dry dimethylformamide (100 mL), cooled to 0° C and sodium azide (1.286 g, 19.78 mmol) added. After stirring for 2 hours and warming to room temperature, the reaction was diluted with dichloromethane and washed with water and brine. The residue left after evaporation was chromatographed (6:1 hexane:ethyl acetate) to give **122** (985 mg, 87%) as a colorless oil, $[\alpha]_D$ -26.9° (*c* 0.5, $CHCl_3$), R_f 0.58 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 5.80 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 4.61 (dd, 1 H $J_{1,2}$ 4 Hz, $J_{2,3}$ 2 Hz, H-2), 4.35 (dt, 1 H, $J_{5,6}$ 6.5 Hz, $J_{4,5}$ 5.5 Hz, H-5), 4.08 (dd, 1 H, $J_{5,6}$ 6.5 Hz, $J_{6a,6b}$ 8.5 H-6a), 3.95 (dd, 1 H, $J_{2,3}$ 2 Hz, $J_{3,4}$ 5.5 Hz, H-3), 3.87 (dd, 1 H, $J_{5,6}$ 6.5 Hz, $J_{6a,6b}$ 8.5 Hz, H-6b), 3.83 (t, 1 H, $J_{4,5} = J_{3,4}$ 5.5 Hz, H-4), 1.57, 1.44, 1.38, 1.36 (s, 3 H, $CO_2(C\underline{H}_3)_2$). ^{13}C NMR ($CDCl_3$): δ 114.41, 110.09 ($\underline{C}O_2(CH_3)_2$), 104.86 (C-1), 85.76 (C-4), 83.13 (C-2), 74.58 (C-5), 65.59 (C-6), 65.56 (C-3), 27.52, 26.90, 26.37, 25.20 ($CO_2(\underline{C}H_3)_2$). IR ($CHCl_3$), 2108.05 cm^{-1}, N_3.

Anal. Calcd for $C_{12}H_{19}N_3O_5$ (285.30): C, 50.52; H, 6.71; N, 14.73. Found: C, 50.37; H, 6.44; N, 14.56.

1,2,4,6-tetra-O-acetyl-3-azido-3-deoxy-D-galactopyranose (**123**). Azide **122** (985 mg, 3.45 mmol), was dissolved in 90% trifluoroacetic acid (10 mL) and stirred at room temperature for 15 min. The reaction was evaporated in vacuo and the resulting oil dissolved in pyridine (30 mL) and then cooled to 0° C. To this solution was added dropwise acetic anhydride (20 mL) and the reaction stirred overnight, warming to room temperature. Evaporation of the solvent followed by chromatography (3:1 hexane:ethyl acetate) gave the product **123** (727 mg, 56%) as a mixture of anomers (α:β 1:1), R_f 0.23 (3:1 hexane:ethyl acetate). Partial ^{1}H NMR ($CDCl_3$): δ 6.16 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1α), 5.50 (d, 1 H, $J_{1,2}$ 8 Hz, H-1β).

2,4,6-tri-O-acetyl-3-azido-3-deoxy-α-D-galactopyranosyl bromide (**124**). Compound **123** (663 mg, 1.78 mmol), was dissolved in dichloromethane (40 mL) and ethyl acetate (4 mL). Titanium tetrabromide (1 g, 2.72 mmol) was added and the reaction stirred at room temperature for three days. The reaction was quenched by adding sodium acetate (1 g) and stirring for 1 hour, diluted with dichloromethane and extracted with water. The organic layer was filtered, evaporated and chromatographed (3:1 hexane:ethyl acetate) to give the product **124** (585 mg, 84%) as an oil R_f 0.36 (3:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 6.70 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.56 (dd, 1 H, $J_{3,4}$ 2.5 Hz, $J_{4,5}$ 1 Hz, H-4), 4.95 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{1,2}$ 3.5 Hz, H-2), 4.41 (t, 1 H, $J_{5,6}$ 6 Hz, H-5), 4.19 (dd, 1 H, $J_{5,6a}$ 6.5 Hz, $J_{6a,6b}$ 11 Hz, H-6a), 4.14 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{1,2}$ 3.5 Hz, H-3), 4.05 (dd, 1 H, $J_{5,6b}$ 6.5 Hz, $J_{6a,6b}$ 11 Hz, H-6b), 2.18, 2.16, 2.07 (s, 3 H, acetate $\underline{C}H_3$).

Octyl 2,4,6-tri-O-acetyl-3-azido-3-deoxy-β-D-galactopyranoside (**125**). Compound **124** (644 mg, 1.74 mmol), was glycosylated as described for the conversion of **109** to **110**, using silver triflate (440 mg, 1.71 mmol), collidine (104 µL, 0.86 mmol) and n-octanol (710 µL, 4.5 mmol) in dichloromethane (10 mL) containing crushed 3 Å molecular sieves (2.5 g). After eight hours the reaction was quenched with collidine (200 µL), filtered and washed with 2 M HCl, water, bicarbonate and brine. After evaporation, the residue was chromatographed (3:1 hexane: ethyl acetate) to give the product **125** (320 mg, 63%) as an oil, $[\alpha]_D$ - 15.0° (*c* 1.0, $CHCl_3$), R_f 0.32 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 5.42 (dd, 1 H, $J_{3,4}$ 1 Hz, $J_{4,5}$, H-4), 5.17 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10.5 Hz, H-2), 4.44 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.13 (d, 2 H, $J_{5,6}$ 7 Hz, H-6a, H-6b), 3.83-3.93 (m, 2 H, $OC\underline{H}_2CH_2$, H-5), 3.58 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{2,3}$ 10.5 Hz, H-3), 3.47 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.19, 2.13, 2.06 (s, 3 H, acetate CH_3), 1.50-1.65 (m, 2 H, $OCH_2C\underline{H}_2$), 1.25-1.40 (m, 10 H, octyl CH_2). 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).^{13}C NMR ($CDCl_3$): δ 170.47, 170.08, 169.25 (acetate C=O), 101.45 (C-1), 71.67 (C-5), 70.24 ($O\underline{C}H_2CH_2$), 69.87 (C-3), 67.78 (C-4), 61.81 (C-3), 61.57 (C-6), 31.85, 29.47, 29.32, 29.29, 25.87, 22.69 (octyl CH_2), 20.79, 20.73, 20.69 (acetate CH_3), and 14.12 (octyl CH_3).

Anal. Calcd for $C_{21}H_{33}N_3O_8$ (443.49): C, 54.17; H, 7.50; N, 9.47. Found: C, 54.40; H, 7.73; N, 9.38.

Octyl 3-azido-3-deoxy-β-D-galactopyranoside **(126)**. Galactoside **125** (300 mg, 0.68 mmol), was dissolved in methanol (10 mL) and sodium methoxide (30 mg) added. After stirring overnight, the solution was neutralized by the addition of prewashed Amberlite IR 120 (H+) resin. Evaporation of the solvent followed by chromatography (19:1 dichloromethane:methanol) gave the product **126** (194 mg, 90%) as a white solid, $[\alpha]_D$ - 0.8° (c 0.9, CH_3OH), R_f 0.15 (19:1 dichloromethane:methanol). ^1H NMR (CD_3OD): δ 4.26 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.83-3.94 (m, 2 H, H-4, $OC\underline{H}_2CH_2$), 3.63-3.75 (m, 3 H, H-2, H-6a, H-6b), 3.47-3.50 (m, 2 H, H-5, $OC\underline{H}_2CH_2$), 3.28 (dd, 1 H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3 Hz, H-3), 1.57-

1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.45 (m, 10 H, octyl CH_2), and 0.83 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 105.07 (C-1), 77.09 (C-5), 70.88 ($O\underline{C}H_2CH_2$), 70.59 (C-2), 69.40 (C-4), 66.88 (C-3), 62.25 (C-3), 32.95, 30.77, 30.51, 30.35, 27.05, 23.66 (octyl CH_2), and 14.40 (octyl CH_3).

Anal. Calcd for $C_{14}H_{27}N_3O_5$ (317.39): C, 52.98; H, 8.58; N, 13.24. Found: C, 53.23; H, 8.87; N, 13.06.

Octyl 3-azido-4,6-benzylidene-3-deoxy-β-D-galactopyranoside (**127**). Compound **126** (119 mg, 0.38 mmol) and benzaldehyde dimethyl acetal (171 μL, 1.14 mmol) were dissolved in acetonitrile (25 mL) and p-toluenesulfonic acid (5 mg) added. After stirring for 30 minutes the reaction was neutralized with triethylamine, evaporated and chromatographed (3:1 hexane:ethyl acetate), to give **127** (130 mg, 85%) as a white solid, $[\alpha]_D$ +6.9° (*c* 0.5, $CHCl_3$), R_f 0.33 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.31-7.55 (m, 5 H, Ph), 5.58 (s, 1 H, $PhC\underline{H}O_2$), 4.33 (dd, 1 H, $J_{5,6a}$ 1.5 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.30 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.21 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 4.08 (dd, 1 H, $J_{5,6a}$ 1.5 Hz, $J_{6a,6b}$ 11.5 Hz, H-6b), 4.02 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10 Hz, H-2), 3.96 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.49 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.46 (br. s 1 H, H-5), 3.35 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3), 2.72 (br. s, 1 H, 2-OH), 1.57-1.62 (m, 2 H, $OCH_2C\underline{H}_2$), 1.15-1.40 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).^{13}C NMR ($CDCl_3$): δ 137.38 (Ph quaternary), 128.99, 128.16, 126.18 (Ph methine), 103.30 (C-1), 101.15 ($Ph\underline{C}HO_2$), 75.17 (C-4), 70.18 ($O\underline{C}H_2CH_2$), 69.19 (C-2), 68.84 (C-5), 67.28 (C-3) 62.25 (C-6), 31.82, 29.52, 29.40, 29.24, 25.97, 22.66 (octyl CH_2), and 14.10 (octyl CH_3).

Anal. Calcd for $C_{21}H_{31}N_3O_5$ (405.40): C, 62.20; H, 7.71; N, 10.36. Found: C, 62.26; H, 7.87; N, 10.29.

Octyl 3-azido-4,6-benzylidene-3-deoxy-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**128**). Alcohol **127** (118 mg, 0.29 mmol) was fucosylated as described for **41** with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.16 mmol) and tetraethylammonium bromide (67 mg, 0.32 mmol). Column chromatography of the mixture (3:1 hexane:ethyl acetate) gave the disaccharide **128** (130 mg, 55%) as a white solid $[\alpha]_D$ - 22.2° (*c* 0.7, CHCl$_3$), R$_f$ 0.42 (3:1 hexane:ethyl acetate). ^{1}H NMR (CDCl$_3$): δ 7.20-7.58 (m, 20 H, Ph), 5.60 (s, 1 H, PhC$\underline{H}$O$_2$), 5.39 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1), 4.97, 4.88, 4.84, 4.77, 4.72, 4.66 (d, 1 H, J_{gem} 11.5 Hz, PhC$\underline{H}_2$), 4.43 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.27-4.38 (m, 3 H, H-5', H-4, H-6a), 4.06-4.14 (m, 3 H, H-6b, H-2, H-2'), 3.94 (dd, 1 H, $J_{2'3'}$ 10 Hz, $J_{3'4'}$ 2.5 Hz, H-3'), 3.90 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OC$\underline{H}_2$CH$_2$), 3.66 (d, 1 H $J_{3'4'}$ 2.5 Hz, H-4'), 3.50 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3), 3.43 (br. s, 1 H, H-5), 3.39 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OC$\underline{H}_2$CH$_2$), 1.50-1.62 (m, 2 H, OCH$_2$C$\underline{H}_2$), 1.15-1.35 (m, 10 H, octyl C$\underline{H}_2$), 1.10 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH$_3$). ^{13}C NMR (CDCl$_3$): δ 139.09, 138.79, 138.49, 137.27 (Ph quaternary), 128.96, 128.35, 128.32, 128.21, 128.18, 127.60, 127.52, 127.44, 127.40, 126.19 (Ph methine), 102.20 (C-1), 101.18 (Ph$\underline{C}$HO$_2$), 97.16 (C-1'), 79.47 (C-4'), 78.02 (C-3'), 76.29 (C-3), 75.56 (C-2'), 74.77, 73.26, 73.24 (PhC$\underline{H}_2$), 71.21 (C-4), 69.74 (O$\underline{C}$H$_2$CH$_2$), 69.23 (C-6), 66.99 (C-5'), 66.68 (C-5), 64.04 (C-3), 31.87, 29.61, 29.51, 29.33, 26.20, 22.67 (octyl CH$_2$), 16.64 (C-6'), and 14.12 (octyl CH$_3$).

Anal. Calcd for $C_{48}H_{59}N_3O_9$ (822.01): C, 70.14; H, 7.23; N, 5.11. Found: C, 70.22; H, 7.43; N, 5.08.

Octyl 3-amino-3-deoxy-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside (**14**). To a solution of protected disaccharide **128** (63 mg, 0.08 mmol) in 4:1 ethanol:dichloromethane (10 mL), was added 10% palladium on carbon (30 mg) and 2 N HCl (40 μL, 0.08 mmol) and the solution stirred under a flow of hydrogen overnight. After completion of the reaction the catalyst was filtered away and the solvent evaporated. Chromatography (10:4:1 dichloromethane:methanol: ammonium hydroxide) followed by dissolution in water and filtration through a 22 μ filter, gave the product **14**

(21 mg, 60%) as a white solid, R_f 0.20 (10:4:1 dichloromethane:methanol: ammonium hydroxide). ^{1}H NMR (D_2O): 5.04 (d, 1 H, $J_{1'2'}$ 2.5 Hz, H-1'), 4.53 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.23 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 4.00 (d, 1 H, $J_{3,4}$ 3 Hz, H-4), 3.62-3.95 (m, 8 H, H-5, H-6a, H-6b, 2 $OC\underline{H}_2CH_2$, H-2', H-3', H-4'), 3.54 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 5 Hz, H-3), 1.60-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.25-1.40 (10 H, octyl CH_2), 1.23 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (D_2O): 102.69 (C-1), 102.46 (C-1'), 79.92 (C-2), 76.73 (C-5), 72.72 (C-3'), 71.45 ($O\underline{C}H_2CH_2$), 70.46 (C-4'), 69.54 (C-2'), 68.13 (C-5'), 67.56 (C-5'), 61.33 (C-6), 57.00 (C-6), 32.12, 29.93, 29.66, 29.47, 26.16, 23.01 (octyl CH_2), 16.55 (C-6'), and 14.38 (octyl CH_3). FABMS (Cleland): *m/z* = 460 $[M+Na]^+$ and 438 $[M+H]^+$ ($C_{20}H_{39}NO_9$ requires *m/z* = 437).

Octyl 4-azido-2,3,6 tri-O-benzyl-4-deoxy-β-D-galactopyranoside **(131)**. To a solution of octyl 2,3,6-tri-O-benzyl-β-D-glucopyranoside (**86**, 203 mg, 0.36 mmol), in 19:1 dichloromethane:pyridine (10 mL) at 0° C, was added dropwise in dichloromethane (2 mL), triflic anhydride (260 µL, 1.53 mmol). After stirring for 30 min., TLC showed the starting material was gone and a new spot (R_f 0.63, 4:1 hexane:ethyl acetate) appeared. The reaction was then extracted with ice cold 5% hydrochloric acid and water, dried with sodium sulfate and evaporated to an orange liquid. The product was directly dissolved in dry dimethylformamide (10 mL), cooled to 0° C and sodium azide (120 mg, 1.85 mmol) added. After stirring for 15 hours and warming to room temperature, the reaction was diluted with dichloromethane and washed with water and brine. The residue left after evaporation was chromatographed (4:1 hexane:ethyl acetate) to give **131** (157 mg, 74%) as a colorless oil, $[\alpha]_D$ - 4.0° (*c* 0.2, $CHCl_3$), R_f 0.50 (4:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.26-7.44 (m, 15 H, Ph), 4.89 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.75 (s, 2 H, $PhC\underline{H}_2$), 4.55 (s, 2 H, $PhC\underline{H}_2$), 4.72 (d, 1 H, J_{gem} 10.5 Hz, $PhC\underline{H}_2$), 4.30 (m, 1 H, H-1), 3.98 (dd, 1 H, $J_{3,4}$ 3 Hz, $J_{4,5}$ 1 Hz), 3.89 (dt, 1 H, J_{gem} 11 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.52-3.70

(m, 5 H, H-2, H-3, H-5, H-6a, H-6b), 3.46 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.52-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.15-1.45 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.57, 137.90, 137.73 (Ph quaternary), 128.52, 128.47, 128.31, 128.13, 127.94, 127.85, 127.79, 127.65 (Ph methine), 103.84 (C-1), 80.01 (C-3), 79.37 (C-2), 75.38, 73.74, 72.95 ($Ph\underline{C}H_2$), 71.57 (C-5), 70.16 ($O\underline{C}H_2CH_2$), 68.69 (C-6), 60.21 (C-4), 31.85, 29.73, 29.43, 29.26, 26.16, 22.68 (octyl CH_2), and 14.11 (octyl CH_3). IR ($CHCl_3$), 2105.69 cm^{-1}, N_3.

Anal. Calcd for $C_{35}H_{45}N_3O_5$ (587.76): C, 71.52; H, 7.72; N, 7.15. Found: C, 71.68; H, 7.99; N, 7.13.

Octyl 4-amino-4-deoxy-β-D-galactopyranoside **(33)**. Galactoside **131** (1.10 g, 1.87 mmol), was dissolved in ethanol (100 mL) and stirred overnight under a flow of hydrogen in the presence of 5% palladium on carbon (550 mg) and 2 N HCl (1.87 mL, 3.74 mmol). Filtration of the catalyst followed by chromatography (10:4:1 dichloromethane:methanol:ammonium hydroxide) gave the product **33** (320 mg, 59%) as a white solid, R_f 0.52 (10:4:1 dichloromethane:methanol:ammonium hydroxide). ^{1}H NMR (D_2O): δ 4.35 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.92 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.67-3.80 (m, 4 H, H-3, H-5, H-6a, H-6b), 3.64 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.44 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, H-2), 3.16 (d, 1 H, $J_{3,4}$ 4 Hz, H-4), 1.55-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (D_2O): δ 104.13 (C-1), 75.45 (C-5), 73.60 (C-3), 71.40 (C-2), 71.34 ($O\underline{C}H_2CH_2$), 61.94 (C-6), 52.42 (C-4), 32.29, 29.93, 29.76, 29.67, 26.24, 23.12 (octyl CH_2), and 14.42 (octyl CH_3). FABMS (Cleland): 292 $[M+H]^+$ ($C_{14}H_{29}NO_5$ requires $m/z = 291$).

Octyl 4-deoxy-4-trifluoroacetamido-β-D-galactopyranoside **(132)**. Compound **33** (270 mg, 0.93 mmol), was dissolved in methanol (75 mL) and S-ethyl-trifluorothioacetate (400 μL, 3.13 mmol) added at 0° C. The reaction was stirred overnight and the solvent evaporated. Chromatography (19:1 dichloromethane: methanol) gave the product **132** (280 mg, 78%) as oil, $[\alpha]_D$ - 20.0° (c 0.3, $CHCl_3$), R_f 0.54 (19:1 dichloromethane:methanol). ^1H NMR (CD_3OD): δ 4.46 (dd, 1 H, $J_{3,4}$ 4.5 Hz, $J_{4,5}$ 1 Hz, H-4), 4.26 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.91 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.74 (dd, 1 H, $J_{3,4}$ 4.5 Hz, $J_{2,3}$ 9.5 Hz, H-3), 3.70 (ddd, 1 H, $J_{5,6a}$ 5.5 Hz, $J_{5,6b}$ 6.5 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.48-3.63 (m, 3 H, H-5, H-6b, $OC\underline{H}_2CH_2$), 3.44 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.5 Hz, H-2), 1.55-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 160.01 (q, $J_{C=O,F}$ 37 Hz, C=O), 117.49 (q, $J_{C,F}$ = 287 Hz, $\underline{C}F_3$), 105.16 (C-1), 75.06 (C-5), 73.22 (C-3), 72.55 (C-2), 71.19 ($O\underline{C}H_2CH_2$), 62.01 (C-6), 52.80 (C-4), 32.94, 30.81, 30.50, 30.36, 27.08, 23.66 (octyl CH_2), and 14.40 (octyl CH_3).

Anal. Calcd for $C_{16}H_{28}F_3NO_6$ (387.40): C, 49.61; H, 7.29; N, 3.62. Found: C, 49.60; H, 7.37; N, 3.58.

Octyl 3,6-di-O-benzoyl-4-deoxy-4-trifluoroacetamido-β-D-galactopyranoside **(133)**. Compound **132** (120 mg, 0.31 mmol) and dibutyl tin oxide (154 mg, 0.62 mmol) were refluxed in dry benzene (30 mL) overnight through a column of 4 Å molecular sieves. The solution was cooled to room temperature and crushed 4 Å molecular sieves (500 mg) and benzoyl chloride (108 µL, 0.93 mmol) were added. After stirring overnight, the solvent was evaporated and the residue chromatographed to give the product **133**, (140 mg, 76%), as a white solid $[\alpha]_D$ - 45.3° (*c* 0.9, $CHCl_3$), R_f 0.27 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.80-8.18 (m, 4 H, Ph), 7.31-7.63 (m, 6 H, Ph), 6.86 (d, 1 H, $J_{NH,CH}$ 10 Hz, $N\underline{H}$), 5.32 (dd, 1 H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 4.5 Hz, H-3), 4.90 (dd, 1 H, $J_{NH,CH}$ 10 Hz, $J_{3,4}$ 4.5 Hz, H-4), 4.59 (dd, 1 H, $J_{5,6a}$ 6.5 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.48 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.27 (dd, 1 H, $J_{5,6b}$ 6.5 Hz, $J_{6a,6b}$ 11.5 Hz, H-6b), 4.18 (dt, 1 H, $J_{5,6a} = J_{5,6b}$ 6.5 Hz, $J_{4,5}$ 1 Hz, H-5), 3.89 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.78 (ddd, 1 H, $J_{1,2}$ 7.5 Hz,

$J_{2,3}$ 10.5 Hz, $J_{2,2\text{-OH}}$ 3 Hz, H-2), 3.58 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.81 (d, 1 H, $J_{2,2\text{-OH}}$ 3 Hz, 2-OH), 1.55-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2). 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 165.95 (C=O, ester), 157.60 (q, 1 C, $J_{C=O,F}$ 38 Hz, C=O), 133.49, 129.79 (Ph methine), 129.21, 129.09, (Ph quaternary), 128.55, 128.46 (Ph methine), 115.73 (q, 1 C, $J_{C,F}$ 288 Hz, $\underline{C}F_3$), 103.78 (C-1), 73.15 (C-5), 70.98 ($O\underline{C}H_2CH_2$), 70.67 (C-3), 70.31 (C-2), 61.72 (C-6), 49.08 (C-4), 31.60, 29.55, 29.31, 29.22, 25.88, 22.65 (octyl CH_2), and 14.09 (octyl CH_3).

Anal. Calcd for $C_{30}H_{36}F_3NO_8$ (595.61): C, 60.50; H, 6.09; N, 2.35. Found: C, 60.26; H, 6.08; N, 2.38.

Octyl 3,6-di-O-benzoyl-4-deoxy-4-trifluoroacetamido-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside **(134)**. Alcohol **133** (130 mg, 0.22 mmol), silver triflate (497 mg, 1.94 mmol, dried for 1 hour over phosphorus pentoxide), and 4-methyl 2,6 di-t-butyl pyridine (318 mg, 1.55 mmol) were stirred in dichloromethane (5 mL) with ground 4 Å molecular sieves (500 mg) for 15 min. The solution was cooled to -78° C and freshly prepared 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.29 mmol) was added dropwise in dichloromethane (5 mL). The solution was allowed to warm to room temperature. Although after four hours TLC showed the presence of starting alcohol, the reaction had stopped and therefore was quenched with collidine and the solution diluted with dichloromethane. The organic solution was washed with 2 M HCl, water, bicarbonate and brine. Column chromatography of the residue left after solvent evaporation (6:1 hexane:ethyl acetate) gave disaccharide **134**, (93 mg, 42%) as oil, $[\alpha]_D$ - 40.3° (*c* 0.3, $CHCl_3$), R_f 0.49 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 6.80-8.10 (m, 25 H, Ph), 6.75 (d, 1 H, $J_{NH,CH}$ 9.5 Hz, $N\underline{H}$), 5.52 (dd, 1 H, $J_{3,4}$ 4.5 Hz, $J_{2,3}$ 9,5 Hz, H-3), 5.34 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.90-4.99 (m, 2 H, H-4, $PhC\underline{H}_2$), 4.76 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.55-4.68 (m, 4 H, 4 $PhC\underline{H}_2$), 4.20-4.34 (m, 4 H, H-1, H-6a, H-6b, H-5'), 4.15 (t, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 4.02 (dd, 1

H, $J_{1,2}$ 7.0 Hz, $J_{2,3}$ 9.5 Hz, H-2), 3.92 (dd, $J_{1'2'}$ 3.5 Hz, $J_{2'3'}$ 9.5 Hz, H-2'), 3.82-3.90 (m, 2 H, $OC\underline{H}_2CH_2$, H-3'), 3.64 (d, 1 H, $J_{3'4'}$ 2 Hz, H-4'), 3.51 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.60-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl $C\underline{H}_2$), 1.13 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), 0.88 (t, 3H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 165.94, 165.18 (C=O, ester), 157.87 (C=O, amide), 138.83, 137.77, (Ph quaternary), 133.61, 133.48, 129.83, 129.69 (Ph methine), 128.95, 128.70, (Ph quaternary), 128.58, 128.42, 128.35, 128.24, 128.14, 127.72, 127.64, 127.46, 127.39 (Ph methine), 102.65 (C-1), 97.58 (C-1'), 79.32 (C-4'), 77.82 (C-3'), 75.56 (C-2'), 74.86 ($PhC\underline{H}_2$), 74.78 (C-2), 73.29, 72.70 ($PhC\underline{H}_2$), 72.00 (C-3), 70.69 ($O\underline{C}H_2CH_2$), 70.31 (C-5), 66.95 (C-5'), 61.65 (C-6), 49.16 (C-4), 31.86, 29.67, 29.41, 29.31, 26.14, 22.69 (octyl CH_2), 16.64 (C-6'), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{57}H_{64}F_3NO_{12}$ (1012.13): C, 67.64; H, 6.37; N, 1.38. Found: C, 67.67; H, 6.27; N, 1.45.

Octyl 4-amino-4-deoxy-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside (**15**). To a solution of protected disaccharide **134** (82 mg, 0.08 mmol) in methanol (10 mL), 10% palladium on carbon (45 mg) was added and the solution stirred under a flow of hydrogen overnight. After completion of the reaction the catalyst was filtered away and the solvent evaporated. The residue was redissolved in methanol (20 mL), 1 M NaOH (1 mL) added and the reaction stirred for 5 hours. The solution was neutralized with Amberlite IR 120 H (+) resin, evaporated and the product further purified as described for **2** to give the product **15** (27 mg, 77%) as a white solid. ^1H NMR (D_2O): δ 5.25 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.44 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.31 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.50-3.97 (m, 10 H, H-2, H-3, H-5, H-6a, H-6b, 2 $OC\underline{H}_2CH_2$, H-2', H-3', H-4'), 3.10 (d, 1 H, $J_{3,4}$ 4.5 Hz, H-4), 1.58-1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.23-1.45 (10 H, octyl CH_2), 1.18 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (D_2O): δ 102.06 (C-1), 99.81 (C-1'), 77.10 (C-2), 74.93 (C-5), 74.07 (C-3), 73.71 (C-3'), 72.12 (C-4'), 70.49 ($O\underline{C}H_2CH_2$), 69.83 (C-2'), 68.57 (C-

5′), 66.83 (C-5), 61.27 (C-6), 51.89 (C-4), 31.57, 29.41, 29.11, 28.95, 25.75, 22.39 (octyl CH_2), 15.83 (C-6′), 13.74 (octyl CH_3). FABMS (Cleland): *m/z* = 460 $[M+Na]^+$ and 438 $[M+H]^+$ ($C_{20}H_{39}NO_9$ requires *m/z* = 437).

Octyl 2-O-allyl-3,4-di-O-benzyl-6-deoxy-6-phthalimido-β-D-galactopyranoside **(135)**. Compound **57** (238 mg, 0.46 mmol), triphenylphosphine (244 mg, 0.932 mmol) and phthalimide (107 mg (0.727 mmol) were dissolved dry tetrahydrofuran (10 mL). To this solution was added diethylazodicarboxylate (160 µL 0.93 mmol) was added and the reaction stirred overnight. The solvent was evaporated and the residue chromatographed (3:1 hexane:ethyl acetate) to give the product **135** (272 mg, 91%) as an oil, $[\alpha]_D$ + 15.8° (*c* 0.8, $CHCl_3$), R_f 0.49 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.65-7.90 (m 4 H Ph phthalimido), 7.20-7.40 (m, 10 H, Ph), 5.96 (1 H, H_c allyl), 5.27 (1 H, H_a allyl), 5.14 (1 H, H_b allyl), 5.04, 4.83, 4.74, 4.73 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.39 (1 H, H_d allyl), 4.22 (1 H, H_e allyl), 4.18 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.09 (dd, 1 H, $J_{5,6a}$ 8 Hz, $J_{6a,6b}$ 13.5 Hz, H-6a), 3.61-3.79 (m, 4 H, $OC\underline{H}_2CH_2$, H-2, H-4, H-5), 3.55 (dd, 1 H, $J_{5,6b}$ 4.5 Hz, $J_{6a,6b}$ 13.5 Hz, H-6b), 3.45 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 2.5 Hz, H-3), 3.29 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.40-1.55 (m, 2 H, $OCH_2C\underline{H}_2$), 1.12-1.30 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 168.06 (C=O), 138.56, 138.48 (Ph quaternary), 135.37 ($CH_2{=}\underline{C}HCH_2O$), 133.96 (Ph methine), 131.97 (Ph quaternary), 128.63, 128.35, 128.19, 127.60, 127.56, 123.22 (Ph methine), 116.51 ($\underline{C}H_2{=}CHCH_2O$), 103.77 (C-1), 82.34 (C-3), 79.06 (C-2), 74.23, 73.86 ($Ph\underline{C}H_2$), 73.77 (C-5), 73.48 ($CH_2{=}CH\underline{C}H_2O$), 71.50 (C-4), 69.97 ($O\underline{C}H_2CH_2$), 38.93 (C-6), 31.75, 29.52, 29.23, 29.18, 25.95, 22.61 (octyl CH_2), and 14.07 (octyl CH_3).

Anal. Calcd for $C_{39}H_{47}NO_7$ (641.81): C, 72.98; H, 7.38; N, 2.19. Found: C, 72.65; H, 7.26; N, 2.19.

Octyl 3,4-di-O-benzyl-6-deoxy-6-phthalimido-β-D-galactopyranoside **(136)**. To a solution of **135** (315 mg, 0.49 mmol) dissolved in 7:3:1 ethanol:benzene:water

(15 mL), tris(triphenylphosphine) rhodium (I) chloride (123 mg, 0.133 mmol) and 1,4 diazobicyclo[2.2.]octane (45 mg, 0.40 mmol) were added and the solution refluxed for 24 hours. The solvent was evaporated and the residue dissolved in 9:1 acetone water (20 mL). Mercuric oxide (5 mg) and mercuric chloride (1.5 g) were added and stirring continued at room temperature overnight. The reaction was then diluted with dichloromethane and washed with saturated potassium iodide, water and brine. Evaporation of the organic layer followed by chromatography (3:1 hexane:ethyl acetate) gave **136** (190 mg, 65%) as a white solid, $[\alpha]_D$ + 19.1° (*c* 0.5, $CHCl_3$), R_f 0.24 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.70-7.90 (4 H, Ph phthalimido), 7.20-7.45 (m, 10 H, Ph), 5.03, 4.80, 4.75, 4.73 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.13 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.11 (dd, 1 H, $J_{5,6a}$ 7.5 Hz, $J_{6a,6b}$ 13.5 Hz, H-6a), 4.00 (ddd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.5 Hz, $J_{2,2\text{-}OH}$ 2 Hz, H-2), 3.79 (d, 1 H, $J_{3,4}$ 3 Hz, H-4), 3.58-3.75 (m, 3 H, H-5, H-6b, $OC\underline{H}_2CH_2$), 3.42 (dd, 1 H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3 Hz, H-3), 3.34 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.41 (d, 1 H, $J_{2,2\text{-}OH}$ 2 Hz, 2-OH), 1.40-1.59 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.45 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): 168.02 (C=O), 138.29, 138.05 (Ph quaternary), 133.97 (Ph methine), 131.89 (Ph quaternary), 128.49, 128.43, 128.20, 127.72, 127.60, 123.21 (Ph methine), 103.10 (C-1), 82.31 (C-3), 74.28 ($Ph\underline{C}H_2$), 73.17 (C-5), 72.81 ($Ph\underline{C}H_2$), 71.83 (C-4), 71.26 (C-2), 69.68 ($O\underline{C}H_2CH_2$), 38.89 (C-6), 31.70, 29.42, 29.19, 29.11, 25.80, 22.56 (octyl CH_2), and 14.03 (octyl CH_3).

Anal. Calcd for $C_{36}H_{43}NO_7$ (601.74): C, 71.86; H, 7.20; N, 2.33. Found: C, 71.96; H, 7.30; N, 2.36.

Octyl 3,4-di-O-benzyl-6-deoxy-6-phthalimido-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside **(137)**. Alcohol **136** (100 mg, 0.17 mmol) was fucosylated as described for **41** with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 0.825 mmol) and tetraethylammonium bromide (38 mg, 0.18 mmol). Column chromatography of the mixture (3:1 hexane:ethyl acetate) gave the disaccharide **137**

(132 mg, 79%) as an oil $[\alpha]_D$ - 54.2° (*c* 0.3, $CHCl_3$), R_f 0.30 (3:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.65-7.90 (m, 4 H, Ph phthalimido), 7.00-7.40 (m, 25 H Ph), 5.68 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.46-4.98 (m, 10 H, 10 $PhC\underline{H}_2$), 4.42 (q, 1 H, $J_{5',6'}$ 6.5 Hz, H-5'), 4.25-4.37 (m, 2 H, H-1, H-2), 4.10 (dd, 1 H, $J_{5,6a}$ 7 Hz, $J_{6a,6b}$ 13 Hz, H-6a), 4.04 (dd, 1 H, $J_{1',2'}$ 3.5 Hz, $J_{2',3'}$ 10 Hz, H-2'), 3.94 (dd, 1 H, $J_{2'3'}$ 10 Hz, $J_{3'4'}$ 2.5 Hz, H-3'), 3.85 (d, 1 H, $J_{3',4'}$ 2 Hz, H-4'), 3.57-3.76 (m, 45 H, H-3, H-4, H-5, H-6b, $OC\underline{H}_2CH_2$), 3.23 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.36-1.49 (m, 2 H, $OCH_2C\underline{H}_2$), 1.10-1.30 (13 H, octyl CH_2, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): 168.05 (C=O), 133.99 (Ph methine), 131.99 (Ph quaternary), 138.92, 138.80, 138.30, 138.23, 137.90 (Ph quaternary), 128.63, 128.43, 128.23, 128.07, 127.99, 127.33, 127.20, 126.35, 123.23 (Ph methine), 102.01 (C-1), 97.21 (C-1'), 84.73 (C-3), 79.62 (C-4'), 78.07 (C-3'), 75.73 (C-2'), 74.70, 74.10, 72.93, 72.65 ($Ph\underline{C}H_2$), 72.12 (C-3), 72.05 (C-5), 71.62 (C-4), 71.58 ($Ph\underline{C}H_2$), 69.40 ($O\underline{C}H_2CH_2$), 66.21 (C-5'), 39.01 (C-6), 31.82, 29.58, 29.39, 29.28, 26.19, 22.63 (octyl CH_2), 16.52 (C-6'), and 14.09 (octyl CH_3).

Anal. Calcd for $C_{63}H_{71}NO_{11}$-H_2O (1036.28): C, 73.04; H, 7.10; N, 1.35. Found: C, 73.27; H, 7.03; N, 1.29.

Octyl 3,4-di-O-benzyl-6-deoxy-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-6-trifluoroacetamido-β-D-galactopyranoside **(138)**. Disaccharide **137** (257 mg, 0.25 mmol) was dissolved in methanol (10 mL) and hydrazine acetate (690 mg, 7.5 mmol) added. The solution was refluxed for 3 hours, then another portion of hydrazine acetate was added (690 mg, 7.5 mmol) and refluxing continued for a total of 24 hours. The solution was cooled, diluted with dichloromethane and washed with water and brine, the crude product showed no phthalimido signal in the ^{1}H-NMR. The product was not further purified, but was dissolved in dimethylformamide (3 mL) and triethylamine (20 µL) added. The solution was cooled to -30° C and then S-ethyl-trifluorothioacetate (160 µL, 1.25 mmol) added dropwise in dimethylformamide (2 mL). The reaction was stirred overnight coming to room temperature. Dilution with dichloromethane followed by extraction with water and brine gave an oil that was chromatographed (6:1 hexane:ethyl acetate) to give the disaccharide **138** (173 mg, 70%) as an oil, R_f 0.32 (6:1 hexane:ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.02-7.44 (m, 25 H Ph), 6.17 (br. s, 1 H, N$\underline{H}$), 5.65 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.96, 4.91 (d, 1 H, J_{gem} 11.5 Hz, PhC$\underline{H}_2$), 4.60-4.80 (m, 5 H, 5 PhC$\underline{H}_2$), 4.57 (m, 2 H, 2 PhC$\underline{H}_2$), 4.50 (d, 1 H, J_{gem} 11.5 Hz, PhC$\underline{H}_2$), 4.41 (m, 2 H, H-1, H-5'), 4.20 (t, 1 H, $J_{1,2}$ 8 Hz, H-2), 4.06 (dd, 1 H, $J_{1'2'}$ 3.5 Hz, $J_{2'3'}$ 10 Hz, H-2), 3.97 (dd, 1 H, $J_{3'4'}$ 2.5 Hz, $J_{2',3'}$ 10 Hz, H-3'), 3.82 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OC$\underline{H}_2$CH$_2$), 3.68-3.74 (m, 3 H, H-4, H-6a, H-6b), 3.66 (d, 1 H, $J_{3',4'}$ 2.5 Hz, H-4'), 3.34-3.57 (m, 3 H, H-3, H-5, OC$\underline{H}_2$CH$_2$), 1.47-1.58 (m, 2 H, OCH$_2$C$\underline{H}_2$), 1.20-1.35 (10 H, octyl CH$_2$), 1.14 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH$_3$).

Octyl 6-deoxy-6-amino-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(16)**. The protected disaccharide **138** (165 mg, 0.17 mmol) was dissolved in methanol (10 mL), 5% palladium on carbon (100 mg) added and the solution stirred under a flow of hydrogen overnight. The catalyst was filtered away, and the solvent evaporated. The product was redissolved in methanol (10 mL) and 1 M NaOH (1 mL) added. After stirring overnight the product was neutralized with Amberlite IR-120 H(+) resin and purified as described for **2** to give **16** (45 mg, 61%) as a white solid. ^{1}H NMR (D_2O): δ 5.27 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.49 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.36 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5), 3.76-3.99 (m, 6 H, H-2', H-3', H-4, H-5, H-2, OC$\underline{H}_2$CH$_2$), 3.52-3.73 (m, 3 H, H-4', H-3, OC$\underline{H}_2$CH$_2$), 2.97 (dd, 1 H, $J_{5,6a}$ 8 Hz, $J_{6a,6b}$ 13 Hz, H-6a), 2.84 (dd, 1 H, $J_{5,6b}$ 4 Hz, $J_{6a,6b}$ 13 Hz, H-6b), 1.57-1.70 (m, 2 H, OCH$_2$C$\underline{H}_2$), 1.21-1.41 (10 H, octyl CH$_2$), 1.11 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH$_3$). ^{13}C NMR (D_2O): δ 102.20 (C-1), 99.98 (C-1'), 76.36 (C-2), 74.35 (C-5), 72.70 (C-3'), 71.50 (O$\underline{C}$H$_2$CH$_2$), 71.29 (C-4'), 70.36 (C-2'), 69.05 (C-4), 67.58 (C-5'), 40.88 (C-6), 31.93, 29.70, 29.40, 29.26, 26.22, 22.83 (octyl CH$_2$), 16.22 (C-

6'), and 14.20 (octyl CH_3). FABMS (Cleland): *m/z* = 460 $[M+Na]^+$ and 438$[M+H]^+$ ($C_{20}H_{39}NO_9$ requires *m/z* = 437).

Octyl 3-acetamido-3-deoxy-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(17)**. Octyl 3-amino-3-deoxy-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside (**14**, 1 mg, 2.28 μmol) was dissolved in methanol (500 μL). Acetic anhydride (1μL) and sodium bicarbonate (2 mg) were added and the reaction stirred for 2 hours, at which point TLC indicated quantitative conversion of the amino derivative to the acetamido derivative. After completion of the reaction, the solvent was evaporated and the product purified by redissolution in water and then passing the solution through a Waters C_{18} Sep-Pak cartridge. The cartridge was washed with water and then the product eluted with methanol. The methanol eluant was evaporated, the residue redissolved in water, filtered through a 0.22 μM filter and lyophilized to give **17**, as a white solid. ^1H NMR (D_2O): δ 5.02 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.58 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.33 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 4.15 (dd, 1 H $J_{2,3}$ 10 Hz, $J_{3,4}$ 3 Hz, H-3), 3.92 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.64-3.85 (m, 7 H, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4', $OC\underline{H}_2CH_2$), 3.60 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10 Hz, H-2), 2.04 (s, 3 H, acetate CH_3), 1.57-1.71 (m, 2 H, $OCH_2C\underline{H}_2$), 1.23-1.49 (m, 10 H, octyl CH_2), 1.20 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.86 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (D_2O): δ 175.06 (C=O), 102.83 (C-1), 100.37 (C-1'), 78.68 (C-2), 75.12 (C-5), 72.70 (C-3'), 71.37 ($O\underline{C}H_2CH_2$), 70.31 (C-4), 68.79 (C-2'), 68.71 (C-4'), 67.73 (C-5'), 61.65 (C-6), 55.60 (C-3), 31.92, 29.67, 29.35, 29.22, 26.10 (octyl CH_3), 22.84 (acetate CH_3), 22.83 (octyl CH_2), 16.28 (C-6'), and 14.22 (octyl CH_3). FABMS (Cleland):*m/z* = 518 $[M+K]^+$, 502 $[M+Na]^+$ and 480 $[M+H]^+$ ($C_{22}H_{41}NO_{10}$ requires *m/z* = 479).

Octyl 4-acetamido-4-deoxy-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(18)**. Octyl 4-amino-4-deoxy-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside (**15**, 1 mg, 2.28 μmol) was acetylated in quantitative yield (by TLC) as described above for the preparation of compound **17**, to provide the product **18** as a white solid. ^1H NMR

(D_2O): δ 5.23 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.51 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.31-4.39 (m, 2 H, H-5', H-4), 4.05 (dd, 1 H $J_{2,3}$ 10 Hz, $J_{3,4}$ 4.5 Hz, H-3), 3.63-3.99 (m, 7 H, 2 $OC\underline{H}_2CH_2$, H-4, H-5, H-2', H-3', H-4'), 3.50 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.0 Hz, H-2), 3.63 (dd, 1 H, $J_{6a,6b}$ 12.5 Hz, $J_{5,6b}$ 5 Hz, H-6a), 3.57 (dd, 1 H, $J_{6a,6b}$ 13 Hz, $J_{5,6b}$ 7.5 Hz, H-6b), 2.07 (s, 3 H, acetate CH_3), 1.56-1.69 (m, 2 H, $OCH_2C\underline{H}_2$), 1.24-1.39 (m, 10 H, octyl CH_2), 1.23 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.86 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (D_2O): δ 176.05 (C=O), 102.61 (C-1), 100.09 (C-1'), 77.18 (C-2), 74.78 (C-5), 73.69 (C-3), 72.76 (C-3'), 71.73 ($O\underline{C}H_2CH_2$), 70.36 (C-4'), 69.09 (C-2'), 67.63 (C-5'), 61.56 (C-6), 51.78 (C-4), 31.95, 29.79, 29.37, 29.26, 26.20, 22.86 (octyl CH_2), 22.73 (acetate CH_3), 16.29 (C-6'), and 14.25 (octyl CH_3). FABMS (Cleland): *m/z* = 402 $[M+Na]^+$ and 480 $[M+H]^+$ ($C_{22}H_{41}NO_{10}$ requires *m/z* = 479).

Octyl 6-acetamido-6-deoxy-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside **(19)**. Octyl 6-amino-6-deoxy-2-O-(α-L-fucopyranosyl)-β-D-galactopyranoside (**16**, 1 mg, 2.28 μmol) was acetylated in quantitative yield (by TLC) as described above for the preparation of compound **17**, to provide the product **19** as a white solid. ^1H NMR (D_2O): δ 5.25 (d, 1 H, $J_{1'2'}$ 3.5 Hz, H-1'), 4.45 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.34 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.63-3.92 (m, 8 H, 2 $OC\underline{H}_2CH_2$, H-3, H-4, H-5, H-2', H-3', H-4'), 3.56 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 8.5 Hz, H-2), 3.44 (dd, 1 H, $J_{6a,6b}$ 13 Hz, $J_{5,6b}$ 5 Hz, H-6a), 3.42 (dd, 1 H, $J_{6a,6b}$ 13 Hz, $J_{5,6b}$ 8 Hz, H-6b), 2.00 (s, 3 H, acetate CH_3), 1.55-1.65 (m, 2 H, $OCH_2C\underline{H}_2$), 1.23-1.37 (m, 10 H, octyl CH_2), 1.20 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.86 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C-NMR (D_2O): δ 175.26 (C=O), 102.27 (C-1), 100.14 (C-1'), 77.25 (C-2), 74.62 (C-5), 73.38 (C-3'), 72.76 (C-4'), 71.42 ($O\underline{C}H_2CH_2$), 70.39 (C-3), 70.11 (C-2'), 69.14 (C-4), 67.60 (C-5'), 40.68 (C-6), 31.95, 29.76, 29.35, 29.26, 26.15, 22.85 (octyl CH_2), 22.66 (acetate CH_3), 16.23 (C-6'), and 14.23 (octyl CH_3). FABMS (Cleland)*m/z* = 518 $[M+K]^+$, 502 $[M+Na]^+$ and 480 $[M+H]^+$ ($C_{22}H_{41}NO_{10}$ requires *m/z* = 479).

Octyl 2,3,4-tri-O-acetyl-α-L-arabinopyranoside (**140**). Silver triflate (1.140 g, 4.43 mmol, dried in vacuo over phosphorus pentoxide for 1 hour), was stirred with collidine (400 µL, 2.96 mmol) and n-octanol (1.4 mL, 8.87 mmol) in dichloromethane (10 mL) containing crushed 3 Å molecular sieves (1.0 g) under nitrogen at -30° C for 20 minutes. To this solution was added dropwise 2,3,4 tri-O-acetyl arabinopyranosyl bromide[173] (**139**, 1.0 g, 2.96 mmol) in dichloromethane (5 mL). The reaction was stirred under nitrogen and warmed to room temperature. After stirring for 90 min the reaction was quenched with collidine (400 µL), filtered and evaporated. The residue was then taken up in dichloromethane and washed with water, then 5% HCl, bicarbonate and finally brine. After solvent evaporation, the resulting clear oil was chromatographed (4:1 hexane:ethyl acetate) to give the product **140** (791 mg, 69%) as an oil, $[\alpha]_D$ + 4.2° (*c* 0.93, $CHCl_3$), R_f 0.42 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 5.26 (br. s, 1 H, H-4), 5.16 (dd, 1 H, $J_{2,3}$ 9.0 Hz, $J_{1,2}$ 7.0 Hz, H-2), 5.04 (dd, 1 H, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.41 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.02 (dd, 1 H, $J_{5a,5b}$ 12.5 Hz, $J_{4,5a}$ 3.5 Hz, H-5a), 3.85 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.63 (dd, 1 H, $J_{5a,5b}$ 12.5 Hz, $J_{4,5a}$ 1.5 Hz, H-5a), 3.45 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.13, 2.06, 2.03 (s, 3 H, acetate CH_3), 1.50-1.64 (m, 2 H, $OCH_2C\underline{H}_2$), 1.19-1.39 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 170.31, 170.14, 169.36 C=O), 100.95 (C-1), 70.22 (C-4), 69.77 ($O\underline{C}H_2CH_2$), 69.28 (C-3), 67.73 (C-2), 63.05 (C-5), 31.79, 29.44, 29.28, 29.23, 25.88, 22.62 (octyl CH_2), 20.90, 20.74, 20.66 (acetate CH_3), and 14.06 (octyl CH_3).

Anal. Calcd for $C_{19}H_{32}O_8$ (388.46): C, 58.75; H, 8.30. Found: C, 58.90; H, 8.50.

Octyl α-L-arabinopyranoside (**38**). Arabinoside **140** (791 mg, 2.04 mmol), was dissolved in methanol (50 mL) and sodium methoxide (20 mg) added. After stirring for 1 hour, the solution was neutralized by the addition of acetic acid. The solvent was evaporated and the residue was chromatographed (19:1 dichloromethane:methanol) to give the product **38** (480 mg, 90%) as a white solid, $[\alpha]_D$ - 6.0° (*c* 0.98, CH_3OH). ^1H NMR (CD_3OD): δ 4.09 (m, 1 H, H-1), 3.65-3.80 (m, 3 H, H-5a, H-4, $OC\underline{H}_2CH_2$), 3.37-3.48 (m, 4 H, H-5b, H-3, H-2, $OC\underline{H}_2CH_2$), 1.45-1.58 (m, 2 H, $OCH_2C\underline{H}_2$), 1.12-1.34 (m, 10 H, octyl CH_2), and 0.80 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.81 (C-1), 74.30 (C-4), 72.42 (C-3), 70.61 ($O\underline{C}H_2CH_2$), 69.61 (C-2), 66.75 (C-5), 33.00, 30.80, 30.54, 30.40, 27.15, 23.71 (octyl CH_2), and 14.43 (octyl CH_3).

Anal. Calcd for $C_{13}H_{26}O_5$ (262.37): C, 59.52; H, 9.99. Found: C, 59.66; H, 10.01.

Octyl 3,4-benzylidene-α-L-arabinopyranoside **(141)**. Compound **38** (311 mg, 1.19 mmol) and benzaldehyde dimethyl acetal (534 μL, 3.56 mmol) were stirred with 3 Å molecular sieves (1 g) and p-toluenesulfonic acid (40 mg) in acetonitrile (20 mL). After 1 hour the reaction was neutralized with triethylamine, the solvent evaporated and the remaining residue chromatographed (6:1 hexane:ethyl acetate). Both diastereomers were present in product **141** in a 4:1 ratio (352 mg, 85%) and were easily separable by chromatography. The slower moving diastereomer was predominant and thus was fully characterized and used, $[\alpha]_D$ + 14.9° (*c* 1.0, $CHCl_3$), R_f 0.32 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.33-7.56 (m, 5 H, Ph), 5.94 (s, 1 H, $PhC\underline{H}O_2$), 4.19-4.35 (m, 4 H, H-5a, H-4, H-3, H-1), 3.80-3.98 (m, 2 H, H-5b, $OC\underline{H}_2CH_2$), 3.70 (ddd, 1 H, $J_{1,2} = J_{2,3}$ 7.5 Hz, $J_{2,2\text{-}OH}$ 2.5 Hz, H-2), 3.49 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 2.41 (d, 1 H, $J_{2,2\text{-}OH}$ 2.5 Hz, 2-OH), 1.55-1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.19-1.49 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 137.03 (Ph quaternary), 129.39, 128.35, 126.72 (Ph methine), 104.64 (C-1), 101.97 ($PhC\underline{H}O_2$), 77.96 (C-4), 75.06 (C-3), 73.77 (C-2), 69.55 ($O\underline{C}H_2CH_2$), 62.27 (C-5), 31.79, 29.59, 29.35, 29.19, 25.98, 22.62 (octyl CH_2), and 14.06 (octyl CH_3).

Anal. Calcd for $C_{20}H_{30}O_5$ (350.46): C, 68.55; H, 8.63. Found: C, 68.12; H, 8.66.

Octyl 3,4-benzylidene-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-α-L-arabinopyranoside **(142)**. Alcohol **141** (140 mg, 0.40 mmol) and tetraethylammonium bromide (92 mg, 0.44 mmol) were dissolved in dichloromethane (3 mL) and dimethylformamide (0.5 mL) containing crushed 4 Å molecular sieves (2.5 g) and the solution stirred overnight. To this slurry was added freshly prepared 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**42**, 1.60 mmol) in dichloromethane (3 mL) and the reaction allowed to stir for 2 days. Methanol (1 mL) was added and stirring continued for thirty minutes and then the reaction was filtered and taken to dryness. The residue was chromatographed (4:1 hexane:ethyl acetate) to give **142** (172 mg, 55%) as an oil, $[\alpha]_D$ - 58.8° (c 0.25, $CHCl_3$), R_f 0.47 (3:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.45 (m, 20 H, Ph), 5.88 (s, 1 H, $PhC\underline{H}O_2$), 5.48 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1'), 4.97, 4.91, 4.79, 4.73, 4.72, 4.66 (d, 1 H, J_{vic} 11.5 Hz, $PhC\underline{H}_2$), 4.48 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.29-4.43 (m, 2 H H-5a, H-4), 4.17 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.90-4.12 (m, 4 H, H-2', H-3', H-2, H-3), 3.85 (dd, 1 H, $J_{5a,5b}$ 13.5 Hz, $J_{4,5b}$ 5 Hz, H-5b), 3.78 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.68 (d, 1 H, $J_{3'4'}$ 2.5 Hz, H-4'), 3.39 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.45-1.60 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.35 (m, 10 H, octyl CH_2), 1.10 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 139.06, 138.62, 138.45, 136.51 (Ph quaternary), 129.42, 128.49, 128.38, 128.28, 128.23, 128.12, 128.02, 127.81, 127.50, 127.34, 126.82 (Ph methine), 104.85 (C-1), 104.57 ($Ph\underline{C}HO_2$), 100.53 (C-1'), 79.40 (C-4'), 78.88 (C-3), 77.85 (C-4), 76.40 (C-2), 76.15 (C-3'), 74.76 ($Ph\underline{C}H_2$), 74.66 (C-2'), 73.36, 72.67 ($Ph\underline{C}H_2$), 68.76 ($O\underline{C}H_2CH_2$), 66.23 (C-5'), 61.99 (C-5), 31.80, 29.74, 29.38, 29.26, 26.16, 22.61 (octyl CH_2), 16.53 (C-6'), and 14.07 (octyl CH_3).

Anal. Calcd for $C_{48}H_{58}O_9$ (778.99): C, 74.01; H, 7.51. Found: C, 73.87; H, 7.81.

Octyl 2-O-(α-L-fucopyranosyl)-α-L-arabinopyranoside **(20)**. The protected disaccharide **142** (130 mg, 0.16 mmol) was dissolved in methanol (5 mL), 5% palladium on carbon (60 mg) added and the solution stirred under a flow of hydrogen for 15 hours. After completion of the reaction, the catalyst was filtered away and the solvent evaporated. The product was purified by redissolution in water and then passing the solution through a Waters C_{18} Sep-Pak cartridge. The cartridge was washed with water and then the product eluted with methanol. The methanol eluant was evaporated, the residue redissolved in water, filtered through a 0.22 µM filter and lyophilized to give **20** (58 mg, 89%) as a white solid. ^{1}H NMR (CD_3OD): δ 5.06 (d, 1 H, $J_{1'2'}$ 2.5 Hz, H-1'), 4.21 (d, 1 H, $J_{1,2}$ 7 Hz, H-1), 4.17 (q, 1 H, $J_{5'6'}$ 6.5 Hz, H-5'), 3.68-3.80 (m, 3 H, $OC\underline{H}_2CH_2$, H-5a, H-3), 3.61-3.68 (m, 3 H, H-4, H-3', H-2'), 3.57 (dd, 1 H, $J_{1,2}$ 7 Hz, $J_{2,3}$ 10 Hz, H-2), 3.54, (s, 1 H, H-4'), 3.43 (dd, 1 H, $J_{5a,5b}$ 12 Hz, $J_{4,5b}$ 1.5 Hz, H-5b), 3.40 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 1.45-1.66 (m, 2 H, $OCH_2C\underline{H}_2$), 1.17-1.36 (m, 10 H, octyl CH_2), 1.10 (d, 3 H, $J_{5'6'}$ 6.5 Hz, H-6'), and 0.81 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 103.46 (C-1), 101.70 (C-1'), 79.08 (C-2), 74.64 (C-3), 73.77 (C-3'), 71.76 (C-4'), 70.60 ($O\underline{C}H_2CH_2$), 70.56 (C-2'), 69.73 (C-4), 67.88 (C-5'), 66.68 (C-5), 33.03, 30.95, 30.60, 30.42, 27.33, 23.71 (octyl CH_2), 16.76 (C-6'), and 14.41 (octyl CH_3). FABMS (Cleland): m/z = 447 $[M+K]^+$, 421 $[M+Na]^+$ and 409 $[M+H]^+$ ($C_{19}H_{36}O_9$ requires *m/z* = 408).

4. 1.3 SYNTHESIS OF MONOSACCHARIDE ANALOGS

Octyl 2, 3, 6 tri-O-benzyl-4-O-[(methylthio)thiocarbonyl]-β-D-glucopyranoside **(149)**. To a solution of **86** (305 mg, 0.54 mmol) in dry tetrahydrofuran (5 mL) was added sodium hydride (50 mg, 80% in oil, 1.66 mmol) and imidazole (10 mg). After stirring for one hour, carbon disulfide (330 µL, 5.40 mmol) was added and stirring continued for another hour. At this point methyl iodide (101 µL, 1.62 mmol) was added and stirring continued overnight. Evaporation of the solvent gave a yellow liquid which was chromatographed (9:1 hexane:ethyl acetate) to give **149** (216 mg, 61%) as an oil, $[\alpha]_D$ +16.5° (*c* 0.5 $CHCl_3$), R_f 0.65 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.20-7.40 (m, 15 H, Ph), 5.97 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 4.92, 4.74, 4.70, 4.65, 4.57, 4.50 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.46 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.96 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.75 (t, 1 H, $J_{2,3} = J_{4,5}$ 9.5 Hz, H-3), 3.52-3.74 (m, 4 H, H-6a, H-6b, H-5, $OC\underline{H}_2CH_2$), 3.51 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.5 Hz, H-2), 2.51 (s, 3 H, SCH_3), 1.57-1.72 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.45 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 215.60 (C=S), 138.42,138.11 (Ph quaternary), 128.38, 128.34, 128.29, 128.15, 128.13, 127.74, 127.66, 127.61 (Ph methine), 103.52 (C-1), 82.02 (C-3), 81.93 (C-2), 79.37 (C-5), 75.33, 75.07 ($Ph\underline{C}H_2$), 73.77 (C-4), 73.74 ($Ph\underline{C}H_2$), 70.37 ($OCH_2\underline{C}H_2$), 69.80 (C-6), 31.87, 29.81, 29.45, 29.30, 26.22, 22.70 (octyl CH_2), 19.37 (SCH_3), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{37}H_{48}O_6S_2$ (652.91): C, 68.07; H, 7.41; S, 9.82. Found: C 68.19; H, 7.59; S, 9.94.

Octyl 2, 3, 6 tri-O-benzyl-4-deoxy-β-D-xylo-hexopyranoside **(150)**. Compound **149** (144 mg, 0.22 mmol) was dissolved in dry toluene (5 mL) and then tributylstannane (300 µL, 1.1 mmol) and AIBN (30 mg, 0.18 mmol) were added. The solution was heated under reflux for 2 hours. Evaporation of the solvent, followed by chromatography (6:1 hexane:ethyl acetate) gave **150** (98 mg, 82%) as a colorless oil, $[\alpha]_D$ -3.7° (*c* 0.6, $CHCl_3$), Rf 0.60 (4:1 hexane:ethyl acetate). ^1H NMR ($CDCl_3$): δ 7.30-7.50 (m, 15 H, Ph), 4.93, 4.75 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.67 (s, 2 H, $PhC\underline{H}_2$), 4.59, 4.54 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.33 (d, 1 H, $J_{1 2}$ 7.5 Hz, H- 1), 3.93 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OCH_2CH_2), 3.45-3.64 (m, 5 H, H-3, H-5, H-6a, H-6b, OCH_2CH_2), 3.31 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.5 Hz, H-2), 2.11 (ddd, 1 H, $J_{3,4e}$ 5.5 Hz, $J_{4e,5}$ 1.5 Hz, $J_{4e,4a}$ 13 Hz, H-4e), 1.59-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-

1.50 (11 H, octyl CH_2, H-4a), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR ($CDCl_3$): δ 138.88, 138.72,138.21 (Ph quaternary), 128.43, 128.36, 128.29, 128.12,127.72, 127.67, 127.55 (Ph methine), 103.90 (C-1), 82.96 (C-3), 78.31 (C-2), 74.98, 73.59, 72.62 ($Ph\underline{C}H_2$), 72.32 (C-6), 71.01 (C-5), 70.16 ($O\underline{C}H_2CH_2$), 34.06 (C-4), 31.87, 29.88, 29.48, 29.29, 26.23, 22.69 (octyl CH_2), and 14.13 (octyl CH_3).

Anal. Calcd for $C_{35}H_{46}0_5$ (546.75): C, 76.89; H, 8.48. Found: C, 76.92; H, 8.61 .

Octyl 4-deoxy -β-D-xylo-hexopyranoside **(22).** Compound **150** (201 mg, 0.37 mmol), was stirred in methanol (10 mL) with 5% palladium on carbon (100 mg) under hydrogen overnight. The reaction was filtered, the solvent evaporated and the residue chromatographed (19:1 dichloromethane: methanol) to give the product **22** (83 mg, 81%) as a white solid, $[\alpha]_D$ -36.9° (*c* 1.1, CH_3OH), R_f 0.10 (19:1 dichloromethane: methanol). ^1H NMR (CD_3OD): δ 4.17 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.87 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OCH_2CH_2), 3.45-3.62 (m, 5 H, H-3, H-5, H-6a, H-6b, OCH_2CH_2), 3.06 (dd, 1 H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 9.5 Hz, H-2), 1.91 (ddd, 1 H, $J_{3,4e}$ 5 Hz, $J_{4e,5}$ 1.5 Hz, $J_{4e,4a}$ 12-5 Hz, H-4e), 1.54-1.67 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.45 (m, 11 H, octyl CH_2, H-4a), 0.90 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).^{13}C NMR (CD_3OD): δ 104.68 (C-1), 76.93 (C-5), 73.83 (C-3), 72.24 (C-2), 70.84 (OCH_2CH_2), 65.57 (C-6), 36.48 (C-4), 32.99, 30.82, 30.56, 30.40, 27.10, 23.69 (octyl CH_2), and 14.42 (octyl CH_3).

Anal. Calcd for $C_{14}H_{28}O5$ (276.38): C, 60.84; H, 10.21. Found: C, 60.91; H, 10.08.

Octyl 6-deoxy-β-D-galactopyranoside **(23).** Octyl 3,4 di-O-benzoyl-6-deoxy-β-D-galactopyranoside **69** (103 mg, 0.21 mmol),was dissolved in methanol (10 mL) and sodium methoxide (10 mg) added. After stirring overnight, the solution was neutralized by the addition of acetic acid. The solvent was evaporated and the residue chromatographed (19:1 dichloromethane:methanol) to give the product **23** (55 mg,

95%) as a white solid. $[\alpha]_D$ - 20.2° (*c* 0.4 $CHCl_3$). Rf 0.15 (19:1 dichloromethane:methanol). ^1H NMR (CD_3OD): δ 4.18 (m, 1 H, H-1), 3.85 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.58-3.70 (m, 2 H, H-4, H-5), 3.52 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.40-3.50 (m, 2 H, H-2, H-3), 1.54-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.28-1.45 (m, 10 H, octyl CH_2), 1.26 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6), 0.90 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.77 (C-1), 75.17 (C-5), 73.02 (C-3), 72.29 (C-2), 71.80 (C-4), 70.75 ($O\underline{C}H_2CH_2$), 32.97, 30.82, 30.52, 30.37, 27.08, 23.67 (octyl CH_2), 16.74 (C-6), and 14.40 (octyl CH_3).

Anal. Calcd for $C_{14}H_{28}O_5$ (276.38): C, 60.84; H, 10.21. Found: C, 60.95; H, 10.44.

Octyl 4-O-methyl-β-D-galactopyranoside **(28)**. Octyl 3,6 di-O-benzyl-4-O-methyl-β-D-galactopyranoside **104** (48 mg, 0.098 mmol), was dissolved in methanol (5 mL), 5% palladium on carbon (20 mg) added and the reaction was allowed to stir overnight under a flow of hydrogen. The catalyst was filtered away, the solvent evaporated and the residue redissolved in water and then passed through a Sep-Pak, washing first with water and then methanol. The methanol eluant was evaporated, redissolved in water, filtered and lyophilized to give **28** (26 mg, 86%) as a white solid. ^1H NMR (CD_3OD): δ 4.16 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.85 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, OCH_2CH_2), 3.68 (d, 2 H, H-6a, H-6b), 3.55 (s, 3 H, OCH_3), 3.41-3.54 (m, 5 H, H-2, H-3, H-4, H-5, $OC\underline{H}_2CH_2$), 1.55-1.65 (m, 2 H, $OCH_2C\underline{H}_2$), 1.25- 1.42 (10 H, octyl CH_2), 0.90 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.97 (C-1), 80.20 (C-4), 76.51 (C-5), 75.60 (C-2), 72.85 (C-3), 70.90 (OCH_2CH_2), 62.01 (OCH_3), 61.70 (C-6), 32.98, 30.80, 30.54, 30.38, 27.07, 23.69 (octyl CH_2), and 14.40 (octyl CH_3). FABMS (Cleland): 345 $[M+K]^+$, 329 $[M+Na]^+$ and 307 $[M+H]^+$ ($C_{15}H_{30}O_6$ requires *m/z* = 306).

Octyl 6-0-methyl-β-D-galactopyranoside **(29)**. Octyl 3,4-di-0-benzyl-6-O-methyl-β-D-galactopyranoside (**107**, 37 mg, 0.77 mmol), was dissolved in methanol (10 mL) and 5% palladium on carbon (20 mg) added. The reaction was allowed to stir overnight under a flow of hydrogen. After filtration of the catalyst, the product was purified as described for **2** to give **29** (23 mg, quantitative) as a white solid, $R_f = 0.22$ (19:1 dichloromethane:methanol). ^1H NMR (CD_3OD): δ 4.12 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.78 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 4.71 (dd, 1 H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1 Hz, H-4), 3.49-3.58 (m, 3 H, H-5, H-6a, H-6b), 3.35-3.48 (m, 3 H, H-2, H-3, OCH_2CH_2), 3.31 (s, 3 H, OCH_3), 1.50-1.59 (m, 2 H, OCH_2CH_2), 1.16-1.35 (10 H, octyl CH_2), and 0.83 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.96 (C-1), 74.94 (C-5), 74.85 (C-3), 73.02 (C-6), 72.52 (C-2), 70.91 ($O\underline{C}H_2CH_2$), 70.52 (C-4), 59.44 (OCH_3), 33.00, 30.84, 30.54, 30.40, 27.10, 23.70 (octyl CH_2), and 14.40 (octyl CH_3). FABMS (Cleland): 345 $[M+K]^+$, 329 $[M+Na]^+$ and 307 $[M+H]^+$ ($C_{15}H_{30}O_6$ requires *m/z* = 306).

Octyl 3-amino-3-deoxy-β-D-galactopyranoside (**32**). Azido-galactoside **126** (60 mg, 0.15 mmol), was dissolved in ethanol (10 mL) and stirred overnight under a flow of hydrogen in the presence of 5% palladium on carbon (35 mg) and HCl (0.30 mmol). Filtration of the catalyst followed by chromatography (10:4:1 dichloromethane: methanol: ammonium hydroxide) gave the product **32** (31 mg, 72%) as a white solid, R_f 0.55 (10:4:1 dichloromethane: methanol: ammonium hydroxide). ^1H NMR (D_2O): δ 4.47 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.12 (d, 1 H, $J_{3,4}$ 3 Hz, H-4), 3.95 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.74-3.84 (m, 3 H, H-5, H-6a, H-6b), 3.71 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.66 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10.5 Hz, H-2), 3.47 (dd, 1 H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3 Hz, H-4), 1.55-1.70 (m 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (10 H, octyl CH_2), and 0.88 (t, 3H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (D_2O): δ 104.06 (C-1), 77.38 (C-5), 72.36 (C-4), 71.39 (OCH_2CH_2), 69.17 (C-4), 61.82 (C-6), 56.20 (C-3), 31.92, 29.58, 29.28, 29.20, 25.87, 22.84 (octyl CH_2), and 14.23 (octyl CH_3). FABMS (Cleland): 314 $[M+Na]^+$ and 292 $[M+H]^+$ ($C_{14}H_{29}NO_5$ requires *m/z* = 291).

Octyl 6-amino-6-deoxy-β-D-galactopyranoside **(34)**. Monosaccharide **136** (58 mg, 0.096 mmol) was dissolved in methanol (15 mL) and hydrazine acetate (258 mg, 2.88 mmol) added. The solution was refluxed for 3 hours, then another portion of hydrazine acetate was added (258 mg, 2.88 mmol) and refluxing continued for a total of 24 hours. The solution was cooled, diluted with dichloromethane and washed with water and brine. The product was not further purified but was immediately redissolved in methanol and 5% Pd-C (50 mg) and HCl (0.192 mmol) added. The solution was stirred under a flow of hydrogen overnight. After filtration of the catalyst, the product was purified as described for **2** to give **34** (19 mg, 70%) as a white solid. ^{1}H NMR (D_2O): δ 4.33 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.83-3.94 (m, 2 H, H-4, $OC\underline{H}_2CH_2$), 3.41-3.67 (m, 4 H, H-2, H-3, H-5, $OC\underline{H}_2CH_2$), 2.92 (dd, 1 H $J_{5,6a}$ 7.5 Hz, $J_{6a,6b}$ 13 Hz, H-6a), 2.84 (dd, 1 H, $J_{5,6b}$ 5 Hz, $J_{6a,6b}$ 13 Hz, H-4), 1.57-1.71 (m, 2 H, $OCH_2C\underline{H}_2$), 1.21-1.41 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).^{13}C NMR (D_2O): δ 103.46 (C-1), 73.30 (C-5), 71.41 (C-3), 71.17 (C-2), 71.38 (OCH_2CH_2), 69.99 (C-4), 40.85 (C-6), 31.94, 29.55, 29.37, 29.25, 25.93, 22.84 (octyl CH_2), and 14.22 (octyl CH_3). FABMS (Cleland): 314 $[M+Na]^+$ and 292 $[M+H]^+$ ($C_{14}H_{29}NO_5$ requires *m/z* = 291).

Octyl 3-acetamido-3-deoxy-β-D-galactopyranoside **(35)**. Octyl 3-amino-3-deoxy-β-D-galactopyranoside (**32**, 1 mg, 2.28 μmol) was dissolved in methanol (500 μL). Acetic anhydride (1μL) and sodium bicarbonate (2 mg) were added and the reaction stirred for 2 hours, at which point TLC indicated quantitative conversion of the amino derivative to the acetamido derivative. After completion of the reaction, the solvent was evaporated and the product purified by redissolution in water and then passing the solution through a Waters C_{18} Sep-Pak cartridge. The cartridge was washed with water and then the product eluted with methanol. The methanol eluant was evaporated, the residue redissolved in water, filtered through a 0.22 μM filter and lyophilized to give **35**, as a white solid. ^{1}H NMR (CD_3OD): δ 4.19 (d, 1 H, $J_{1,2}$ 7.5

Hz, H-1), 3.82 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), 3.71-3.80 (m, 2 H H-3, H-4) , 3.64 (dd, 1 H, $J_{5,6a}$ 7 Hz, $J_{6a,6b}$ 12 Hz, H-6a), 3.60 (dd, 1 H, $J_{5,6b}$ 5 Hz, $J_{6a,6b}$ 12 Hz, H-6b), 3.39-3.51 (m, 3 H, H-2, H-5, $OC\underline{H}_2CH_2$), 2.03 (s, 3 H, acetate CH_3), 1.58-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.46 (m, 10 H, octyl CH_2), and 0.86 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).

Octyl 4-acetamido-4-deoxy-β-D-galactopyranoside **(36)**. Octyl 4-amino-4-deoxy-β-D-galactopyranoside (**33**, 1 mg, 2.28 μmol) was acetylated in quantitative yield (by TLC) as described above for the preparation of compound **35**, to provide the product **36** as a white solid. ^{1}H NMR (D_2O): δ 4.20 (m, 2 H, H-1, H-4), 4.05 (dd, 1 H $J_{2,3}$ 10 Hz, $J_{3,4}$ 4.5 Hz, H-3), 3.63-3.99 (m, 3 H, $OC\underline{H}_2CH_2$, H-3, H-5), 3.45 (dt, 1 H, J_{gem} 10 Hz, J_{vic} 7 Hz, $OC\underline{H}_2CH_2$), (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.0 Hz, H-2), 3.46 (dd, 1 H, $J_{5,6a}$ 7 Hz, $J_{6a,6b}$ 12 Hz, H-6a), 3.34 (dd, 1 H, $J_{5,6b}$ 5 Hz, $J_{6a,6b}$ 12 Hz, H-6b), 3.20 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.5 Hz, H-2), 2.07 (s, 3 H, acetate CH_3), 1.55-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2), 0.86 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).

Octyl 6-acetamido-6-deoxy-β-D-galactopyranoside **(37)**. Octyl 6-amino-6-deoxy-β-D-galactopyranoside (**34**, 1 mg, 2.28 μmol) was acetylated in quantitative yield (by TLC) as described above for the preparation of compound **35**, to provide the product **37** as a white solid. ^{1}H NMR (D_2O): δ 4.17 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.99 (d, 1 H, $J_{3,4}$ 5 Hz, H-4), 3.86-3.92 (m, 2 H, $OC\underline{H}_2CH_2$, H-3), 3.58-3.78 (m, 3 H, H-5, H-6a, H-6b), 3.21 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10 Hz, H-2), 1.99 (s, 3 H, acetate CH_3), 1.56-1.70 (m, 2 H, $OCH_2C\underline{H}_2$), 1.20-1.40 (m, 10 H, octyl CH_2), and 0.86 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).

4.2 ENZYME KINETICS

4.2.1 A AND B TRANSFERASES

Radiochemical assays were based on modification of a previously described method which takes advantage of the use of hydrophobic acceptors and products to facilitate the removal of unreacted radiolabelled donor from reaction products. Human serum used as a source of the A and B transferases was prepared by allowing freshly drawn blood to clot at room temperature for 2 hours, refrigerating overnight at 4° C and centrifuging to remove blood clots. The serum was then stored frozen at -20°C in 100 µL aliquots until use. Incubations for the A and B assays were carried out in 600 µL plastic microfuge tubes at 37° C.

For the A transferase, all assays were carried out in a total volume of 66 µL with 50 mM sodium cacodylate buffer, pH 6.9, containing 20 mM $MnCl_2$, 30 µM UDP-GalNAc, 0.2 µCi UDP-[6-^{3}H]-GalNAc and 10 µL of human serum containing the A transferase (61 µU/mL serum). Under these conditions, the rate of product formation with the native disaccharide **2** was shown to be linear up to a time of 60 min. Incubations were carried out for 45 min and then quenched by the addition of EDTA (400 µL of a 23 mM solution). The reaction mixtures were transferred to pre-equilibrated C_{18} Sep-Pak cartridges and the unreacted radiolabelled donor removed by washing with dilute ammonia and then water until background counts were obtained. The radiolabelled product was eluted with methanol (1 x 3 mL) and quantitated by liquid scintillation counting. The K_m of **2** was determined to be 1.50 µM under these conditions. Assays to test activity as an acceptor were carried out at concentrations of 2.5 µM. The results are presented in Table 5. To test for inhibitory activity, the potential inhibitor (25 µM) was added to **2** at 2.5 µM. The results are recorded in Table 7. At concentrations of **2** greater than 25 µM, substrate inhibition was observed. K_m determinations for compounds **2**, **5**, and **8** were carried out at the following concentrations: 25, 20, 15, 12.5, 10, 7.5, 6.25, 3.13, 1.56, and 0.78 µM. For compound **11** the K_m was determined over these concentrations: 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 µM. Finally, in determining the K_m of **16**, the substrate concentrations used were: 259, 129.5, 64.8, 32.4, 16.2, 8.1, and 4 µM. K_i

determinations for compound **3** were done with inhibitor concentrations of 25, 50 and 75 µM. For compound **6** inhibitor concentrations of 44, 88 and 132 µM were used.

For the B transferase, assay conditions were identical to those described above except that the solution contained 30 µM UDP-Gal, 0.2 µCi UDP-[6-^{3}H]-Gal, 250 µM ATP and 25 µL of human serum containing the B transferase (12.8 µU/mL serum). Using these conditions, the rate of product formation with the native disaccharide **2** was linear up to a time of 180 min. Incubations were carried out for 120 min. The K_m of **2** was 21.91 µM under these conditions. Assays to test activity as an acceptor were carried out at concentrations of 10 µM. To test for inhibitory activity, the potential inhibitor (100 µM) was added to disaccharide **2** at 10 µM. At concentrations of **2** greater than 50 µM, substrate inhibition was observed. See Tables 5 and 7 for results. In determining K_m's, the following substrate concentrations were used. Compound **2**: 50, 37.5, 25, 18.75, 12.5, 6.25, 3.13, 1.56 µM. Compounds **5** and **8**: 150, 125, 100, 75, 50, 37.5, 25, 18.75, 12.5 µM. Compound **11**: 804.5, 605.4, 402.25, 302.7, 201.1, 100.6, 50.3, 25.1, and 12.5 µM. Compound **16**: 826.4, 630, 413.2, 310, 206.6, 103.3, 51.7, 25.8, and 12.9 µM. Inhibitor concentrations of 10, 20 and 30 µM were used in the determination of the K_i of **3**. In determining the K_i of **6**, inhibitor concentrations of 60, 150 and 200 µM were used. Finally, inhibitor concentrations of 5, 10 and 15 µM were used in the determination of the a K_i for **12**.

Rate data were fit to the Michaelis-Menten equation using unweighted nonlinear regression with the SigmaPlot 4.0 program to estimate the kinetic parameters shown in Table 6. Inhibition constants shown in Table 8 were determined by fitting the data to an equation for competitive inhibition using the SigmaPlot 4.0 program.

4. 2. 2 H-TRANSFERASE

4. 2. 2. 1. CLONING OF THE H-TRANSFERASE

The H-fucosyltransferase used in this study was cloned in a manner similar to that of Larsen at. al.[122] with the plasmid used by Wei et. al.[188] The enzyme used here differed from the one cloned by Larsen et al. in that this enzyme was attached to the fusion protein (Protein A) through the second histidine following the transmembrane domain. In Larsen's clone, Protein A was joined to the first proline following the transmembrane domain (See Fig 17). The beads containing the immobilized cloned protein were stored at 4° C in a buffer of 10 mM Tris containing 150 mM sodium chloride and 25% glycerol, pH 7.4.

4. 2. 2. 2. CLEAVAGE OF ENZYMES FROM BEADS

The entire cleavage procedure was carried out at 4° C. Initially, the immobilized enzyme beads (20μL) were initially washed by mixing them in a 600 μL centrifuge tube with 100 μL of a 1 mM Tris buffer containing 150 mM sodium chloride, pH 8.0. This suspension was spun down in a microcentrifuge and the supernatant aspirated from the tube. The cleavage was carried out by adding, to the resin beads, 50 μL of a buffer containing 0.1M sodium citrate and 0.1 M citric acid, pH 4.4. The beads were quickly mixed in the buffer three times with a micropipette, the solution spun down and the supernatant was directly added to 20 μL of a quench buffer containing 1M Tris, pH 8.2. This cleavage/quenching procedure was repeated five times. Finally, to each of the quenched cleavage solutions was added 70 μL of a storage buffer containing 5 mM $MnCl_2$, 25 mM sodium cacodylate, and 25% glycerol, pH 6.5. The individual fractions were assayed for activity, using the assay described below. Typically between three and five of the cleavage fractions contained active enzyme.

4. 2. 2. 3 ASSAY CONDITIONS

For the H-transferase, all assays were carried out in a total volume of 40 μL with 20 mM HEPES buffer, pH 7.0, containing 20 mM $MnCl_2$, 0.2% BSA, 50 μM GDP-Fuc, 0.05 μCi GDP-[1-^{3}H]-Fuc and 3 μL of the enzyme in the aforementioned storage buffer (1 mU/mL solution). Under these conditions, the rate of product formation with the native monosaccharide 1 was shown to be linear up to a time of 90 min. Incubations were carried out for 60 min and then quenched by the addition of water. The reaction mixtures were transferred to pre-equilibrated C_{18} Sep-Pak cartridges and the unreacted radiolabelled donor removed by washing with water until background counts were obtained. The radiolabelled product was eluted with methanol (1 x 3 mL) and quantitated by liquid scintillation. The K_m of 1 was determined to be 6.4 mM under these conditions. Assays to test activity as an acceptor were carried out at concentrations of 6.4 mM. The results are presented in Table 9. To test for inhibitory activity, the potential inhibitor (6.4 mM) was added to 1 at 6.4 mM. The results are recorded in Table 10. The K_m determination for compound 1, was carried out at the following concentrations: 17.5, 15.0, 10.0, 7.5, 5.0, 2.5, 1.25 mM.

CHAPTER FIVE:

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CHAPTER SIX:

APPENDIX:KINETIC PLOTS

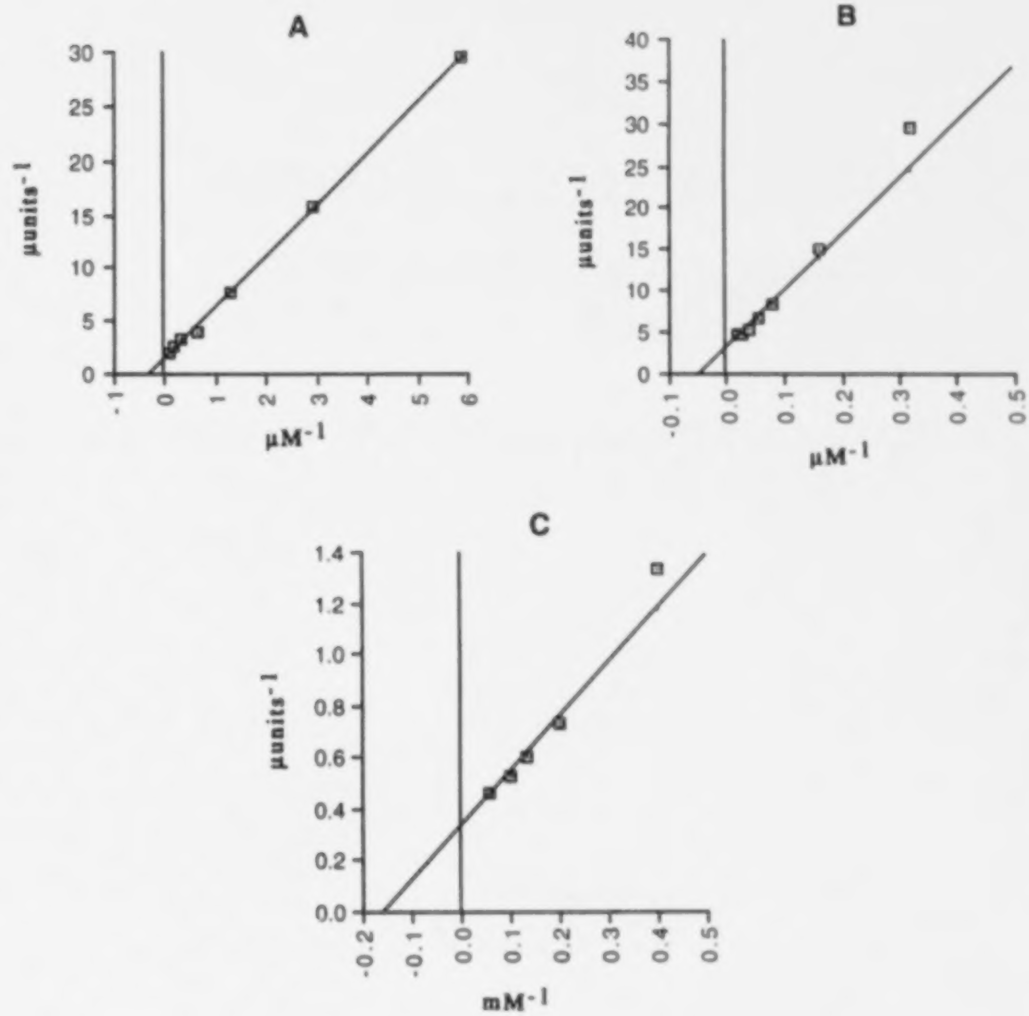

Fig 70. Lineweaver-Burk plots for parent substrates. **A.** Disaccharide **2** with the A-transferase **B**. Disaccharide **2** with the B-transferase. **C.** Monosaccharide 1 with the H-transferase.

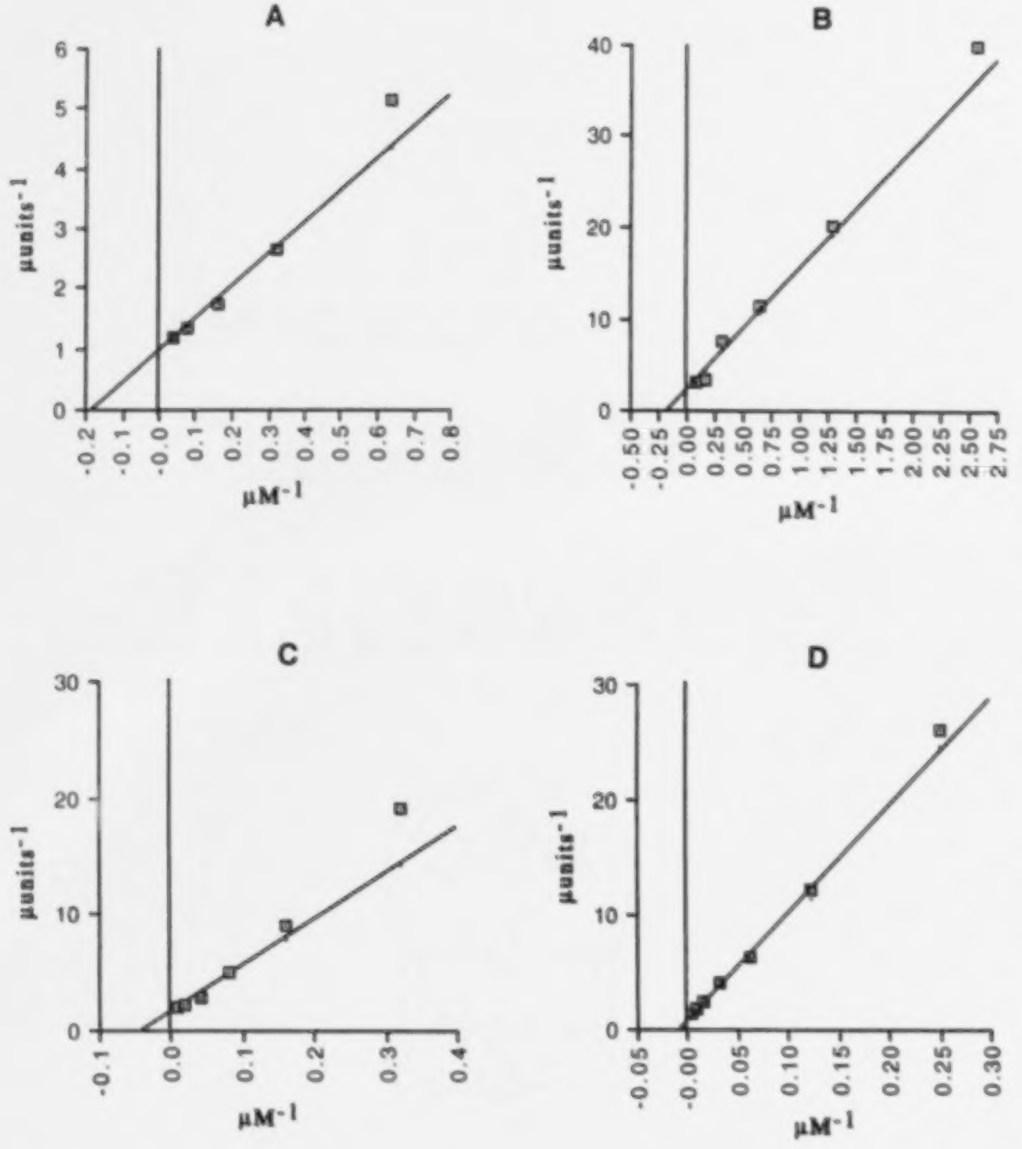

Fig 71. Lineweaver-Burk plots of acceptors with the A-transferase. **A.** 6-deoxy disaccharide 5. **B.** 6-fluoro disaccharide 8. **C.** 6-O-methyl disaccharide 11. **D.** 6-amino disaccharide 16.

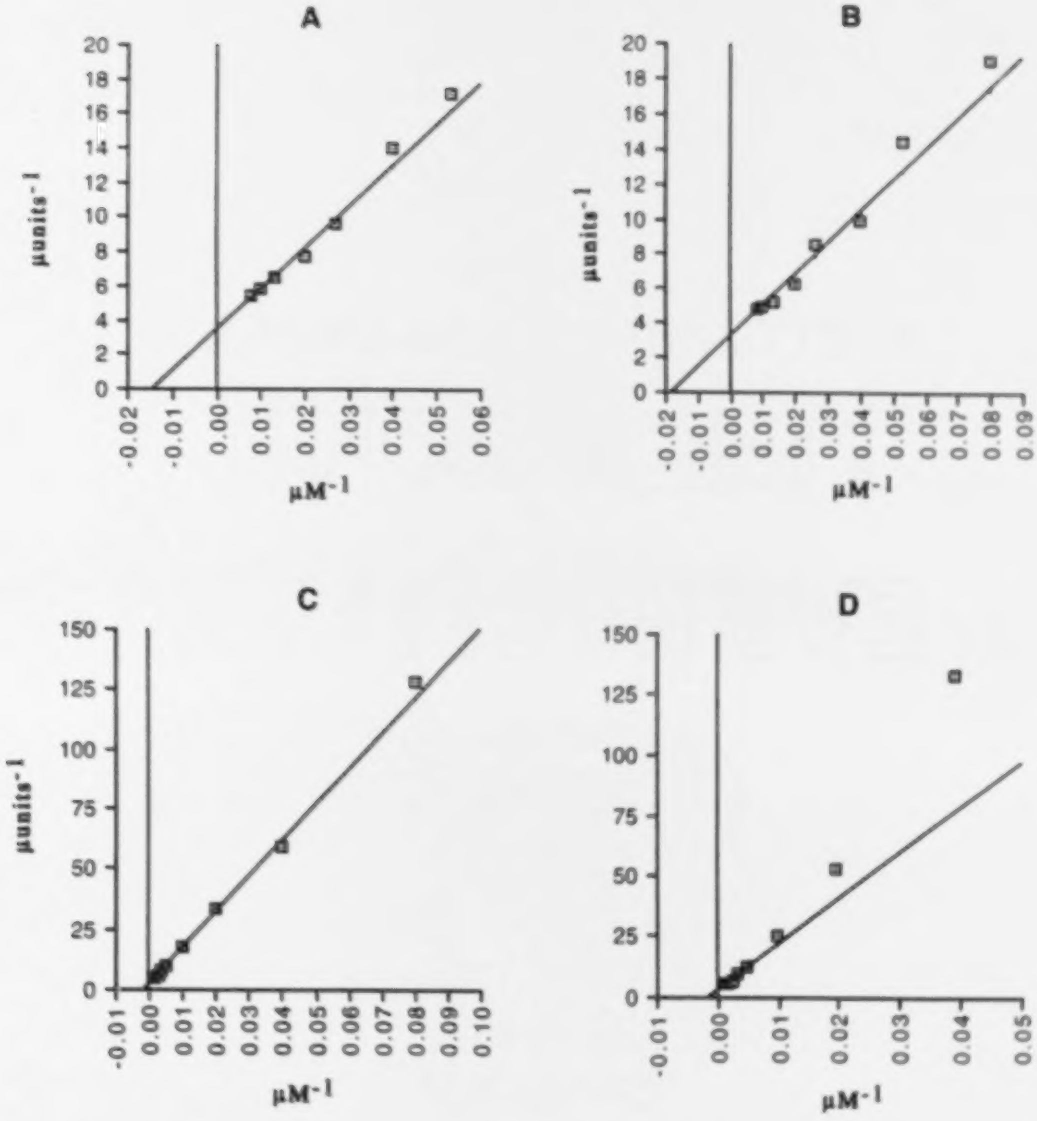

Fig 72. Lineweaver-Burk plots of acceptors with the B-transferase. **A**. 6-deoxy disaccharide 5. **B**. 6-fluoro disaccharide 8. **C**. 6-O-methyl disaccharide 11. **D**. 6-amino disaccharide 16.

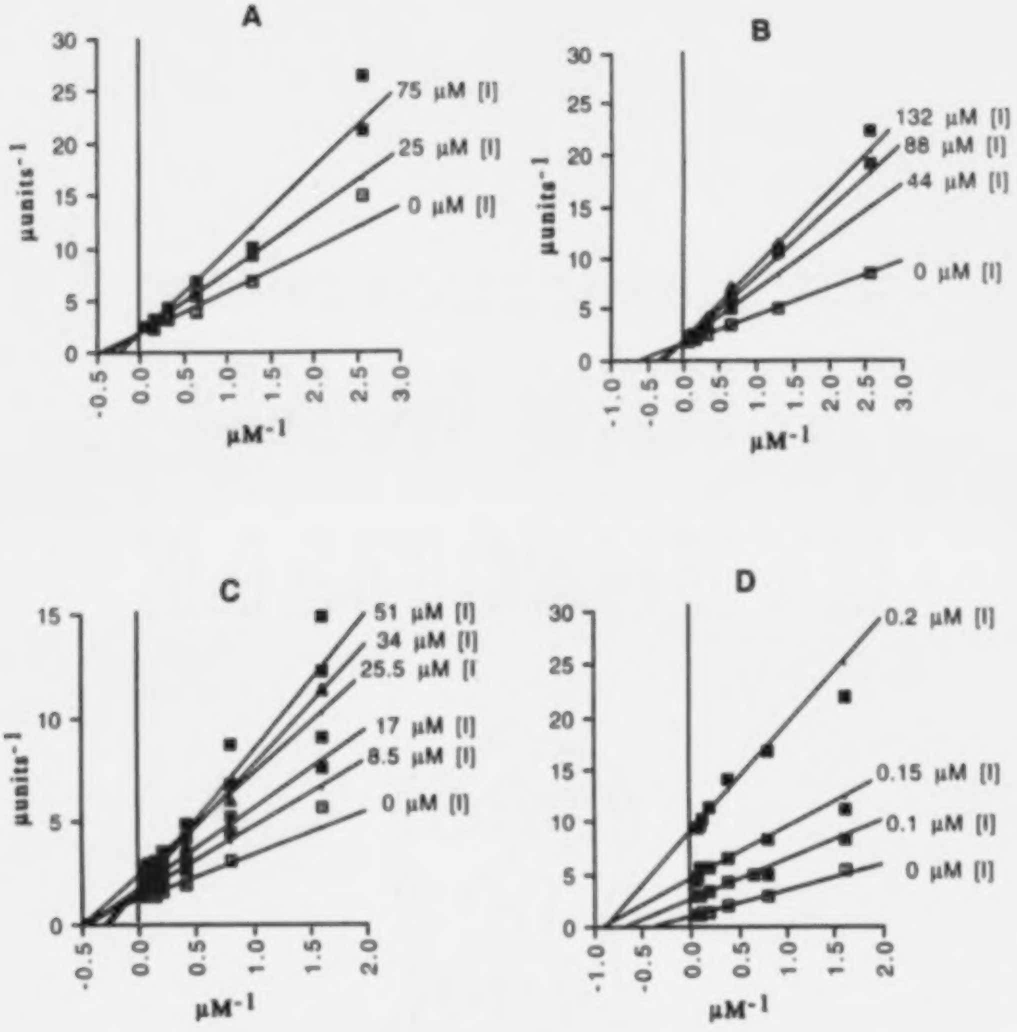

Fig 73. Lineweaver-Burk plots of inhibitors with the A-transferase. **A**. 3-deoxy disaccharide 3. **B**. 3-fluoro disaccharide 6. **C**. 6-epimer 12. **D**. 6-amino disaccharide 14.

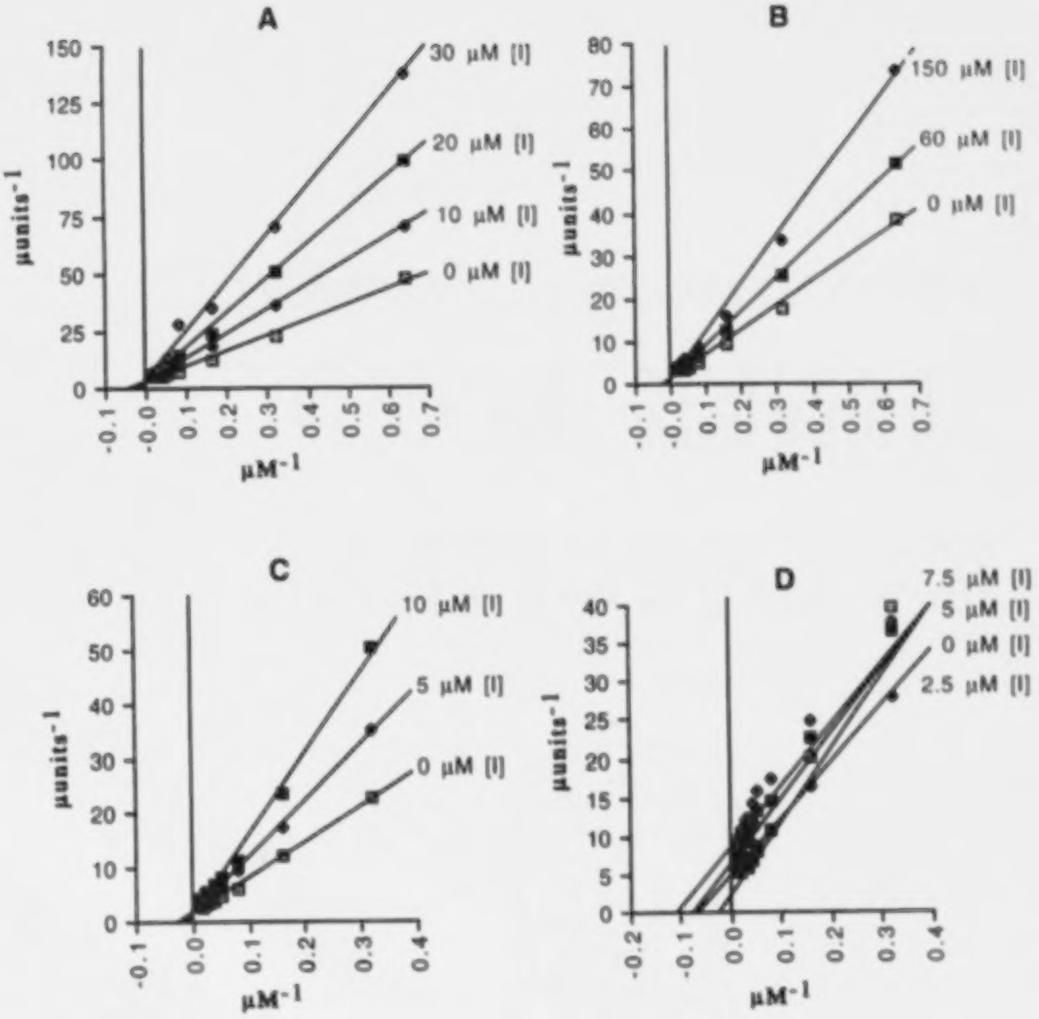

Fig 74. Lineweaver-Burk plots of inhibitors with the B-transferase. **A**. 3-deoxy disaccharide 3. **B**. 3-fluoro disaccharide 6. **C**. 6-epimer 12. **D**. 6-amino disaccharide 14.